National Library of Canada
Bibliothèque nationale du Canada

Canadian Theses Service
Services des thèses canadiennes

Ottawa, Canada
K1A 0N4

CANADIAN THESES
THÈSES CANADIENNES

NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

AVIS

La qualité de cette microfiche dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

Les documents qui font déjà l'objet d'un droit d'auteur (articles de revue, examens publiés, etc.) ne sont pas microfilmés.

La reproduction, même partielle, de ce microfilm est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30. Veuillez prendre connaissance des formules d'autorisation qui accompagnent cette thèse.

THIS DISSERTATION
HAS BEEN MICROFILMED
EXACTLY AS RECEIVED

LA THÈSE A ÉTÉ
MICROFILMÉE TELLE QUE
NOUS L'AVONS REÇUE

NL 339 (r. 86/01)

Canada

National Library of Canada | Bibliothèque nationale du Canada

Ottawa, Canada
K1A 0N4

69

TC –

0-315-22935-7

CANADIAN THESES ON MICROFICHE SERVICE – SERVICE DES THÈSES CANADIENNES SUR MICROFICHE

PERMISION TO MICROFILM – AUTORISATION DE MICROFILMER

• Please print or type – Écrire en lettres moulées ou dactylographier

AUTHOR – AUTEUR

Full Name of Author – Nom complet de l'auteur

Maan Hussien Alie Saad

Date of Birth – Date de naissance
27, 12, 1959

Canadian Citizen – Citoyen canadien
☐ Yes / Oui ☒ No / Non

Country of Birth – Lieu de naissance
Sierra leone
West Africa

Permanent Address – Résidence fixe
9-70 Basic Medical Sciences Bldg, Dept. of pharmacology
U of A, Edmonton, Alta

THESIS – THÈSE

Title of Thesis – Titre de la thèse

Studies on the modulation of airway reactivity in the guinea pig.

Degree for which thesis was presented
Grade pour lequel cette thèse fut présentée
Ph.D

Year this degree conferred
Année d'obtention de ce grade
1985

University – Université
Alberta

Name of Supervisor – Nom du directeur de thèse
J. F. Burka

AUTHORIZATION – AUTORISATION

Permission is hereby granted to the NATIONAL LIBRARY OF CANADA to **microfilm** this thesis and to **lend** or **sell copies of the film.**

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

L'autorisation est, par la présente, accordée à la BIBLIOTHÈQUE NATIONALE DU CANADA de **microfilmer** cette thèse et de **prêter** ou de **vendre des exemplaires du film.**

L'auteur se réserve les autres droits de publication; ni la thèse ni de longs extraits de celle-ci ne doivent être imprimés ou autrement reproduits sans l'autorisation écrite de l'auteur.

ATTACH FORM TO THESIS – VEUILLEZ JOINDRE CE FORMULAIRE À LA THÈSE

Signature

Date
18, July 1985

NL-91 (r. 84/03)

Canada

THE UNIVERSITY OF ALBERTA

STUDIES ON THE MODULATION OF AIRWAY REACTIVITY IN THE GUINEA PIG

By

(C) Maan Saad

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

Doctor of Philosophy

Pharmacology

Edmonton, Alberta

Fall 1985

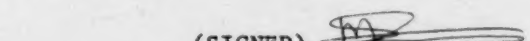
THE UNIVERSITY OF ALBERTA

RELEASE FORM

NAME OF AUTHOR	Maan H.A. Saad
TITLE OF THESIS	Studies on the Modulation of airway reactivity in the guinea pig
DEGREE FOR WHICH THESIS WAS PRESENTED	Doctor of Philosophy
YEAR THIS DEGREE GRANTED	Fall 1985

Permission is hereby granted to THE UNIVERSITY OF ALBERTA LIBRARY to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.

(SIGNED) 

PERMANENT ADDRESS:
10 Ferngate Drive
Withington, Manchester
England
M20 9AX

DATED: July 18 1985

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled 'Studies on the modulation of airway reactivity in the guinea pig' submitted by Maan H.A. Saad in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Pharmacology.

.....

Supervisor

.....

.....

.....

.....

External Examiner

Date June, 10, 1985

ABSTRACT

The effects of immunological sensitization to ovalbumin (OA) were investigated in isolated guinea-pig airway tissues. It was found that the responses and sensitivity of normal and sensitized tracheal spirals and lung parenchymal strips to bronchoconstrictors including leukotrienes (LTs) C_4 and D_4, and bronchodilators, including isoproterenol and vasoactive intestinal peptide (VIP), were similar in both groups of animals. Indomethacin-pretreatment enhanced the responses of trachea to high concentrations of bronchoconstrictors andreduced responses of lung parenchymal strips to bronchodilators.

The bronchodilators stimulated adenylate cyclase activity of normal and sensitized lung parenchyma to similar extents.

It was concluded that immunological sensitization to OA does not induce airway hyperreactivity to bronchoconstrictors or airway hyporeactivity to bronchodilators in guinea-pig airways in vitro. Apparent airway-hyperreactivity of the trachea to bronchoconstrictors may result from removal of the negative modulatory influence of PGE_2 upon treatment of tissues with indomethacin. The reduction in bronchodilator efficacy in lung parenchyma upon indomethacin-pretreatment is unexplained and was not paralleled by a reduction in adenylate cyclase activity. Thus, there is a lack of correlation between bronchodilation and adenylate cyclase activation of lung strips.

It was further demonstrated that calcium ionophore A23187- and AA-induced contractions of airway tissues were similar in both normal

and sensitized tissues. Furthermore, it was found that airway-tissue contraction induced by OA and A23187 was reduced by mepacrine and nordihydroguaiaretic acid (NDGA) pretreatment. These agents inhibit phospholipase A_2 and lipoxygenase-enzyme activity, respectively. The effects of all these stimuli were enhanced by indomethacin-pretreatment of trachea. The effects were similar on both normal and sensitized tissues.

It was concluded that OA and A23187 stimulate AA metabolism via the lipoxygenase pathway. The mediators responsible for OA- A23187-, and AA-induced contractions were isolated and purified by high performance-liquid chromatography and subjected to bioassay and ultraviolet spectrometry studies. The evidence indicated that these mediators included LTC_4 and LTD_4.

Contractions to AA were reduced in calcium-free media and further reduced by EDTA and lanthanum ions. Re-addition of calcium reversed the inhibition. Nitrendipine prevented this reversal. Nitrendipine and verapamil inhibited AA-induced contractions but did not affect LTD_4-induced contractions. Hence it was concluded that extracellular calcium is more important for metabolism of AA to LTs than for smooth muscle contraction induced by the newly synthesized LTs.

Finally, the effects of LTD_4 on the adenylate cyclase activity of lung membranes were investigated. Current evidence indicated that LTD_4 inhibited adenylate cyclase. The effects of LTD_4 were investigated on guinea-pig lung and rat cerebellar adenylate cyclase.

GTPγS-induced inhibition can be used as an index of the inhibitory potential of hormones in various cyclase systems. It was found that GTPγS inhibition of lung cyclase was small relative to inhibition measured in the cerebellar cyclase system. Moreover, LTD_4-induced inhibition of lung cyclase activity was small and erratic. LTD_4 did inhibit the lung and cerebellar enzymes to a marked degree on some occasions. However, the reproducibility of the data was a major problem in these experiments.

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to Dr. J.F. Burka, my supervisor and friend, for his invaluable guidance and support throughout the course of my tenure as a graduate student at the University of Alberta.

I also gratefully acknowledge the council, criticism, and encouragement that I received from the other members of my supervisory committee, viz, Drs. H.P. Baer, D.F. Biggs, and K. Wong. I would especially like to further thank Dr. H.P. Baer for allowing me to perform the cyclase assays in his laboratory, and for his numerous helpful suggestions regarding my work.

I extend my sincere thanks to all my colleagues and friends, too numerous to mention by name, for their moral and technical support. Special thanks are due to Mr. Gus Duchon for drafting some of the figures in this thesis, and to Mrs. Cecilia Kwong and Mrs. Linda Chambers for uncomplainingly typing and retyping successive drafts of the manuscript.

Finally, I wish to thank the Alberta Heritage Foundation for Medical Research for the generous financial support during the course of my studies at the University of Alberta.

Table of Contents

Chapter								Page
1.	INTRODUCTION							1
	1.1	ASTHMA AND AIRWAY HYPERREACTIVITY						2
	1.2	MECHANISMS OF ASTHMA						3
		1.2.1	Humoral and immunological mechanisms in asthma					3
			1.2.1.1	Biochemical events preceding mediator release				4
			1.2.1.2	Chemical mediators in asthma				6
			1.2.1.3	Prostaglandins and the airways				6
				1.2.1.3.1	Prostaglandin biosynthesis			6
				1.2.1.3.2	Effects of PGs on the airways			12
			1.2.1.4	Pharmacology of the leukotrienes				13
				1.2.1.4.1	Leukotriene biosynthesis			15
				1.2.1.4.2	Inhibition of lipoxygenase product synthesis and effects			17
				1.2.1.4.3	Biological sources of lipoxygenase products			18
				1.2.1.4.4	Effects of leukotrienes on the airways			21

Page

1.2.1.4.5 Effects of leukotrienes on the central nervous system . 23

1.2.1.4.6 Leukotriene receptors . . . 24

1.2.1.4.7 Possible intracellular mechanism of action of LTD_4 . 27

1.2.2 Autonomic control of the airways 28

1.2.2.1 Parasympathetic and sympathetic nervous system 28

1.2.2.2 Non-adrenergic non-cholinergic (NANC) inhibitory innervation 30

1.2.3 Basic regulatory processes in the airways 31

1.2.3.1 Adenylate cyclase 32

1.2.3.1.1 Characteristics of adenylate cyclase inhibition 34

1.2.3.2 Cyclic AMP and the airways 35

1.2.3.3 Calcium and the airways 37

1.2.3.3.1 Sources of utilizable calcium in the airways 38

1.2.3.3.2 Calcium channels in smooth muscle 38

1.2.3.3.3 Excitation-contraction coupling in smooth muscle . . 40

1.2.3.3.4 Calcium antagonists in asthma 40

Page

1.3 STUDIES ON MODELS OF ASTHMA: MECHANISMS OF AIRWAY HYPERREACTIVITY 42

1.3.1 Sensitization-dependent changes in pharmacologic receptors 43

1.3.2 Sensitization-dependent changes in cyclic AMP and PGs 46

1.3.3 Sensitization-dependent changes in the role of lipoxygenase metabolites 48

2. RESEARCH OBJECTIVES 50

3. MATERIALS AND METHODS 54

3.1 MATERIALS 55

3.2 METHODS 56

3.2.1 Sensitization of animals and preparation of tissues 56

3.2.2 Concentration-response curves to broncho-constrictors and bronchodilators: Effects of cyclooxygenase and lipoxygenase inhibitors on responses to agonists 59

3.2.3 Responses to antigen, ionophore, A23187 and AA. Effect of AA metabolism inhibitors and calcium modulatory treatments 62

Page

3.2.4 Extraction, initial purification, reverse-phase high performance liquid chromatography, ultraviolet spectrometry and bioassay of leukotrienes released from airway tissues . . . 65

3.2.5 Adenylate cyclase assay procedure 69

3.2.5.1 Preparation of membranes 69

3.2.5.2 Adenylate cyclase assay procedure . . 70

4. RESULTS 73

4.1 RESPONSES OF NORMAL AND SENSITIZED AIRWAY TISSUES TO BRONCHOCONSTRICTORS AND BRONCHODILATORS 74

4.1.1 Contractile responses to bronchoconstrictors . 74

4.1.2 Sensitivity of tracheal and parenchymal strips to bronchoconstrictors 77

4.1.3 Effects of cyclooxygenase and lipoxygenase inhibitors on the responsiveness and sensitivity of airways to bronchoconstrictors 79

4.1.4 Relaxant responses to bronchodilators 89

4.1.5 Effects of indomethacin-pretreatment on drug-induced relaxations 89

4.1.6 Effects of bronchodilators on adenylate cyclase activity in control and indomethacin-pretreated lung membranes 93

Page

4.1.6.1 Effect of indomethacin-pretreatment on adenylate cyclase activation 98

4.2 ANTIGEN-, A23187-, AND AA-INDUCED CONTRACTIONS OF NORMAL AND SENSITIZED AIRWAY TISSUES 98

4.2.1 Effects of indomethacin, NDGA, or mepacrine . . 98

4.2.2 High performance liquid chromatography 111

4.2.3 Bioassay of released LTs 115

4.2.4 Nature of the mediators released from airway tissue after stimulation with AA, A23187 or OA . 119

4.3 ROLE OF CALCIUM IN ARACHIDONIC ACID-INDUCED CONTRACTION OF GUINEA PIG AIRWAYS 119

4.3.1 Effects of calcium and TMB-8 119

4.3.2 Effects of nitrendipine and verapamil 121

4.3.3 Effects of lanthanum and EDTA. Reversal by calcium readdition 122

4.3.4 Concentration-response curves to LTC_4 126

4.4 EFFECTS OF LTD_4 ON LUNG CEREBELLAR ADENYLATE CYCLASE ACTIVITY 128

4.4.1 Initial studies 128

4.4.2 Effects of LTD_4 on lung adenylate cyclase . . . 141

4.4.3 Effects of GTPγS on guinea-pig lung and rat cerebellar adenylate cyclase 154

Page

4.4.4 Effects of GABA and LTD_4 on cerebellar adenylate cyclase activity 164

5. DISCUSSION 175

5.1 Effects of bronchoconstrictors and bronchodilators . . 176

5.2 AA metabolism in guinea-pig airways. Role of calcium 188

5.3 Effects of LTD_4 on lung and cerebellar adenylate cyclase 197

6. BIBLIOGRAPHY 206

List of Tables

Table		Page
1	Mediators in asthma	7
2	Representative sources of lipoxygenase products	19
3	Neurohumoral influences acting on airway smooth muscle (ASM)	29
4	Experimental protocol for the investigation of the effects of mepacrine and NDGA on the contractile effects of A23187, ovalbumin, and AA	63
5	Experimental protocol for Ca^{2+} studies	64
6	Percentage recovery of [^{3}H]-LTC_4 utilizing C_{18}-SEP-PAKs	67
7	Increases in force after the addition of bronchoconstrictors	76
8	Sensitivity of normal and sensitized-airway tissues to bronchoconstrictors	78
9	Effect of inhibitors on tone and percentage change from preincubation tone	80
10	Effect of inhibitors on sensitivity of tracheal spirals to histamine and carbachol	88
11	Sensitivity of airway tissues from normal and sensitized animals to relaxant agents in vitro	91

Page

12 Maximum control relaxations of airway tissues from normal and sensitized guinea pigs after addition of bronchodilators 92

13 Sensitivity of normal and sensitized airway tissues to bronchodilators 95

14 Adenylate cyclase activity of control and indomethacin-pretreated lung membranes 101

15 Release of LTs from guinea-pig airway tissue in the first hour 118

16 Effects of GTP on lung adenylate cyclase in the presence or absence of adenosine deaminase 136

17 Basal adenylate cyclase activities 140

18 GTPγS inhibition of adenylate cyclase activity 156

19 Percentage inhibition of rat cerebellar cyclase basal activity in the presence of Mg^{2+} and Mn^{2+} 170

List of Figures

Figure		Page
1	Arachidonic acid metabolism	11
2	Arachidonic acid metabolism by 5-lipoxygenase	16
3	Length-tension relationships of isolated guinea pig airways	58
4	Elution pattern of LTC_4	68
5	Concentration-response curves to bronchoconstrictors in tracheal spirals and parenchymal strips from normal and ovalbumin-sensitized guinea pigs	75
6	Effects of indomethacin, phenidone and NDGA on responses of normal and sensitized trachea to histamine and carbachol	81
7	Effects of indomethacin, phenidone, and NDGA on responses of normal and sensitized trachea to LTC_4 and LTD_4	83
8	Effects of indomethacin on responses of normal and sensitized trachea to U-44069	84
9	Effects of indomethacin, phenidone, and NDGA on responses of normal and sensitized parenchyma to histamine, carbachol and U-44069	85
10	Effects of indomethacin, phenidone, and NDGA on responses of normal and sensitized parenchyma to LTC_4 and LTD_4	87
11	Relaxant responses to isoproterenol, PGE_2, VIP, and forskolin in normal and sensitized airway tissues	90

Page

13 Effects of indomethacin on the relaxant responses of normal and sensitized parenchymal strips 96

14 Hormonal specificity of the responses of guinea-pig lung parenchymal adenylate cyclase 97

15 Activation of normal and sensitized lung adenylate cyclase by isoproterenol, PGE_2, and VIP 99

16 Effects of indomethacin-pretreatment on lung adenylate cyclase activation induced by forskolin 100

17 Concentration-response curves to A23187 on normal and sensitized lung tracheal strips 102

18 Concentration-response curves to A23187 on normal and sensitized lung parenchymal strips 103

19 Responses of sensitized airway tissues to ovalbumin 105

20 Contractile responses of normal and sensitized trachea to A23187 and ovalbumin 106

21 The responses of normal and sensitized trachea to AA: effects of mepacrine pretreatment 107

22 The responses of normal and sensitized parenchymal strips to AA: effects of mepacrine 108

23 The responses of indomethacin-treated tracheal spirals to AA, A23187, or OA in the presence of mepacrine 109

24 The responses of indomethacin-treated parenchymal strips to AA, A23187, or OA in the presence of mepacrine 110

Page

25 Effects of NDGA on the tracheal response to AA, A23187, or ovalbumin 112

26 Effects of NDGA on the lung parenchymal response to AA, A23187, or ovalbumin 113

27 Typical HPLC chromatograms of released LTs 114

28 Ultraviolet spectra of synthetic and natural LTC_4 116

29 Typical bioassay tracings of natural LTC_4 117

30 The effects of calcium and TMB-8 on AA-induced contractions of airway tissues 120

31 Effects of nitrendipine and verapamil on AA-induced contractions of trachea and parenchyma 123

32 Effects of lanthanum chloride and EDTA on AA-induced contractions of trachea 124

33 Effects of lanthanum chloride and EDTA on AA-induced contractions of parenchyma 125

34 Contractions of trachea and parenchyma to LTC_4 127

35 Time-course of lung adenylate cyclase activation at 20°C . . 129

36 Protein-linearity of lung adenylate cyclase assays 130

37 Activation of lung adenylate cyclase at 30°C 131

38 Stimulation of lung adenylate cyclase at 37°C in the presence of two different Mg^{2+} concentrations 132

39 Stimulation of lung adenylate cyclase at 20°C by forskolin and isoproterenol 133

Page

40 Stimulation of lung adenylate cyclase by GTP in the absence and presence of LTD_4 135

41 The effects of adenosine deaminase on the responses of rat-cerebellar adenylate cyclase to GTP 137

42 The effects of adenosine deaminase on the responses of rat cerebellar cyclase to GTP and adenosine 138

43 Lack of effect of LTD_4 on forskolin or isoproterenol-stimulated lung adenylate cyclase activity 142

44 Effect of LTD_4 on forskolin-stimulated lung adenylate cyclase 143

45 Effect of LTD_4 on lung adenylate cyclase in the absence or presence of NaCl 144

46 Effect of GTP on LTD_4-induced inhibition of lung adenylate cyclase 145

47 Effect of LTD_4 on lung adenylate cyclase activity at 37°C . 147

48 Effect of LTD_4 on lung adenylate cyclase activity in the presence of Mg^{2+} or Mn^{2+} 148

49 Effect of LTD_4 on lung adenylate cyclase activity in the absence or presence of NaCl 149

50 Binding of [^{3}H]-LTC_4 to guinea pig lung membranes at 20°C . 151

51 Inhibition of lung adenylate cyclase activity by Ca^{2+} . . . 152

52 Inhibition of cerebellar adenylate cyclase activity by Ca^{2+} 153

Page

53 Inhibition and stimulation of lung adenylate cyclase activity by GTPγS at 20°C 155

54 Effects of GTPγS on forskolin-stimulated lung adenylate cyclase at 37°C 158

55 Effects of GTPγS on lung and cerebellar cyclase activities at 37°C 159

56 Time course of GTPγS inhibition of cerebellar cyclase activity 160

57 Effect of GTPγS on cerebellar adenylate cyclase activity at 20°C 162

58 Effect of protease inhibitors on lung adenylate cyclase activity 163

59 Effects of GTPγS on cerebellar adenylate cyclase activity in the presence of GABA or LTD_4 165

60 Effects of LTD_4 and GABA on cerebellar adenylate cyclase activity at 20°C 166

61 Inhibition of cerebellar adenylate cyclase activity by GABA at 20°C 168

62 Inhibition of cerebellar adenylate cyclase activity by GABA and GTPγS under various conditions 169

63 Percentage inhibition of cerebellar adenylate cyclase activity by GABA, LTD_4 and LTC_4 at 20°C 172

64 Effects of LTD_4 on cerebellar adenylate cyclase in the presence of GTP 173

ABBREVIATIONS

AA:	Arachidonic acid (5,8,11,14, eicosatetraenoic acid)
AD:	Adenosine deaminase
ASM:	Airway smooth muscle
ATP:	Adenosine Triphosphate
BSA:	Bovine serum albumin
Cyclic AMP:	Cyclic adenosine 3',5',monophosphate
DTT:	Dithiothreitol (cleland's reagent)
EDTA:	Ethylenediaminetetracetic acid
GABA:	γ-aminobutyric acid
Gpp(NH)p:	Guanyl-5'-(β,γ,imino)triphosphate
GTP:	Guanosine triphosphate
GTPγS:	Guanosine-5'-(3-O-thio)triphosphate
HETE:	Hydroxyeicosatetraenoic acid
HPETE:	Hydroperoxyeicosatetraenoic acid
HPLC:	High performance liquid chromatography
IgE and IgG:	Immunoglobulins E and G, respectively
INA:	Isoproterenol
KHS:	Krebs Hensleit solution
LT:	Leukotriene
NANC:	Non-adrenergic non-cholinergic innervation
NDGA:	Nordihydroguaiaretic acid
OA:	ovalbumin

PG:	Prostaglandin
PLA_2:	Phospholipase A_2
RBL-1:	Rat basophillic leukaemia cells
TXA_2:	Thromboxane A_2
VIP:	Vasoactive intestinal polypeptide

1. INTRODUCTION

1.1 ASTHMA AND AIRWAY HYPERREACTIVITY: INTRODUCTION

Bronchial asthma is a complex disease characterized by reversible obstruction of both conducting (large) and distensible (small) airways. The main components of airway obstruction in asthma include airway smooth muscle constriction, smooth muscle hypertrophy and hyperreactivity, mucosal inflammation, goblet cell and mucus gland hypertrophy, mucus hypersecretion and plug formation, and a decreased rate of mucus clearance (Leff, 1982; Wanner, 1983). Pulmonary function tests of asthmatic patients indicates increases in resistance to airflow due to constriction of the larger airways, decreases in compliance due to decreases in distensibility of the smaller airways and increases in residual volume of the lung due to trapping of air in alveolae. Post mortem examinations of patients who died of asthma consistently show that edema and infiltration of inflammatory cells in the bronchial mucosa have occurred (Hogg, 1983) suggesting the development of inflammation.

The etiology of asthma is complex. Factors implicated in its development and pathogenesis include atopy (Cross, 1981), viral and bacterial respiratory tract infections (Welliver, 1983), psychological influences (Herrera & Fialkor, 1981), environmental influences (Hendrick et al. 1981), and a genetic predisposition towards airway hyperreactivity to nonspecific and pharmacological agents. Patients with asthma can be broadly divided into extrinsic and intrinsic groups. In the former group, there is a known causative external

agent (Garland, 1984), as opposed to the latter group, where an external stimulus is not apparent.

1.2 MECHANISMS OF ASTHMA

Asthma is a syndrome that arises as a consequence of immunological, humoral, autonomic and cellular biochemical regulatory mechanisms that operate within the airways. Dysfunctions in these regulatory influences may contribute to the hyperreactivity of airways inherent in asthma.

1.2.1. Humoral and Immunological Mechanisms in Asthma:

Allergic asthma is initiated in susceptible individuals by prior sensitization of components of the immune system with a foreign substance (i.e. allergen) which is usually a protein moeity (Foreman & Lichtenstein, 1980). Subsequent development of specific antigen hypersensitivity then paves the way for the immediate (type 1) hypersensitivity reaction which results in anaphylactic release of mediators when antigen bivalently crosslinks antibodies bound to the effector cells of the immune system (e.g. mast cells, basophils, macrophages (Ishizaka, 1982). Subsequent release of mediators in the vicinity of the airway smooth muscle and other effector cells initiates bronchoconstriction, edema, and mucus accumulation (Austen & Orange, 1975; Eyre & Burka, 1978; Leff, 1982; Lagunoff, 1983).

The mediators primarily involved in airway obstruction include

histamine, prostaglandins (PGs) $F_2\alpha$ and D_2 and leukotrienes (LTs) C_4, D_4 and E_4. The pharmacology of PGs and LTs in the airways is reviewed in sections 1.2.1.3 and 1.2.1.4. Histamine can act directly to contract airway smooth muscle via H_1 receptors or indirectly via irritant receptors in the airways to induce vagally-mediated reflex bronchoconstriction (Gold et al. 1972; Leff, 1982; Biggs, 1984). Histamine can also increase the release of acetylcholine from cholinergic nerve terminals in the airways (Theoharides et al. 1982).

1.2.1.1. Biochemical events preceding mediator release:

The Ishizakas in 1967 showed that the reaginic antibody in human asthma is immunoglobulin E (IgE). This is similar to the situation in rat, mouse, dog, and monkey. In the guinea pig, the reaginic antibody appears to be of the IgG_1 class (Mongar, 1965; Andersson, 1980). However, the guinea pig can be made to produce IgE in addition to IgG_1 (Andersson, 1980). Bryant et al. (1973, 1975) showed that IgG can play a role in human asthma, but that this involves a subgroup of IgG called IgG_4 which has homocytotropic properties. IgE has the capacity to sensitize tissues from normal individuals passively. Details of the structure of IgE have been reviewed (Tada, 1975; Ishizaka, 1976; Metzger, 1979; Foreman & Lichtenstein, 1980; Ishizaka, 1982).

Activation of mast cell mediator release can occur when antigen bivalently crosslinks two IgE molecules via their antigen binding regions (Fab). Other stimuli that can activate mast cells to induce

mediator release include compound 48/80, polymyxin B, dextran, mellitin, formyl-met-leu-phe, C5a and C3a anaphylatoxins, phosphatidic acid, and degranulating peptide from cobra venom. The calcium ionophore A23187 is a useful nonspecific tool in mediator release experiments.

A variety of biochemical processes appear to be important in mast cell activation. These include phospholipid methylation, phosphatidyl inositol (PI) turnover, adenylate cyclase activation and Ca^{2+} entry into the cell. The first three processes appear to be vital for the last process to occur (Metzger, 1979, Hirata & Axelrod, 1980; Ishizaka et al. 1980; Ishizaka et al. 1984).

Histamine secretion by activated mast cells parallels Ca^{2+} uptake (Foreman et al. 1977; Foreman, 1980; Ishizaka et al. 1983). The rise in ionized calcium inside the mast cell induced by various secretagogues serves to activate phospholipase (PL) A_2 (Billah et al. 1980; Lapetina et al. 1981; Wightman et al. 1982) which splits off arachidonic acid (AA) from phosphatidylcholine. Free AA is metabolized by cyclooxygenase and the calcium-dependent lipoxygenase (Jakschik & Lee, 1980) to produce PGs and LTs, respectively. Moreover, calcium is required in the exocytotic release of preformed mediators from the mast cell. The effects of calcium are probably exerted via calmodulin (Cheung, 1980; Steinhardt & Alderton, 1982).

1.2.1.2 Chemical mediators of asthma:

The mast cell is central to the currently held views of the pathogenesis of allergic asthma (Austen & Orange, 1975; Lagunoff, 1983). Upon stimulation, mast cells release a plethora of mediators (summarized in Table 1).

1.2.1.3 Prostaglandins and the airways:

PGs are local modulators of cellular responses. The role of PGs in homeostasis and body functions has received intense attention. The literature has been well reviewed (Moncada & Vane, 1979; Stenson & Parker, 1980; Mcgiff, 1981; Moncada, 1981, Goetzl, 1981; Spannhake et al. 1981; O'Flaherty, 1982; Burka, 1983).

1.2.1.3.1 Prostaglandin biosynthesis:

PGs and thromboxanes (TXs) are derived via cyclooxygenase metabolism of AA (Fig. 1). The bulk of AA in cells is esterified in the 2-acyl position of glycerophospholipids of the cell membrane (see Irvine, 1982). PGs are not stored to any great extent in a tissue. An increase in their levels represents de novo synthesis. It is generally agreed that the first rate limiting step in PG synthesis is the release of bound AA into a free form. This is accomplished by a calcium dependent PLA_2 (see section 1.2.1.1).

Activation of PLA_2 can be induced by mechanical or electrical stimulation (Turker & Zengil, 1976), and by challenge of airway tissue

TABLE 1. Mediators in Asthma

Mediator	Status in tissue	Main Biologic Activity	Comments	Selected References
Histamine	Preformed	Constricts airway smooth muscle, activates irritant receptors leading to vagal reflex contraction of the airways, increases vascular permeability, induces hypotension	Released with heparin, released very rapidly after mast cell activation in the early stages of the immediate-hypersensitivity reaction.	Dale & Laidlaw, (1910). Kazimierczak & Diamant, (1978). Austen & Orange (1975). Theoharides et al. (1982). Biggs, (1984).
5-Hydroxy-tryptamine	Preformed	As histamine	Rodent mast cells	Theoharides et al. (1982).
LTC_4, LTD_4, LTE_4	Newly synthesized from AA via the lipoxygenase pathway	Potent bronchoconstrictors, increase mucus secretion, decrease mucus clearance, stimulate PG synthesis, increase vascular permeability, vasoconstrictors and contractile on a variety of smooth muscles.	Peptidolipid leukotrienes. 1000-10,000 times more potent as bronchoconstrictors than histamine. More potent on small airways than large airways. Shown to be released from human asthmatic lung after antigen challenge.	Kellaway & Trethewie, (1940). Brocklehurst, (1968). Lewis et al. (1980). Morris et al. (1980). Goetzl (1981). Peters et al. (1981). Samuelsson, (1982). O'Flaherty, (1982).

TABLE 1. (Cont'd) Mediators in Asthma

Mediator	Status in tissue	Main Biologic Activity	Comments	Selected References
LTB_4 (5,12-di-HETE)	Newly synthesized from AA via the lipoxygenase pathway	Bronchoconstrictor on small airways, induces TXA_2 synthesis. Chemokinesis and chemotaxis of leucocytes.	Most potent chemokinetic and chemotactic agent for neutrophils and eosinophils.	Borgeat & Samuelsson (1979) Ford-Hutchinson et al. (1980). Sirois et al. (1982). Bray, (1983).
5-HETE		Bronchoconstrictor, stimulates histamine release from mast cells	Modulator of histamine release	Peters et al. (1981).
PGD_2, $PGF_2\alpha$ TXA_2	Newly synthesized from AA via the cyclooxygenase pathway.	Bronchoconstrictors, vasoconstrictors, stimulate irritant receptors, immunomodulators.	PGD_2 is the major PG released from human lung mast cells.	Goetzl, (1981). Spannhake et al (1981). O'Flaherty, (1982).
PGE_2 PGI_2	cyclooxygenase products.	Bronchodilator and bronochoconstrictors depending on dose, vasodilators. Inhibit mediator release via cyclic AMP synthesis.	Important modulators of airway responses to bronchoconstrictors.	Goetzl, (1981). Spannhake, (1981).

TABLE 1. (Cont'd) Mediators in Asthma

Mediator	Status in tissue	Main Biologic Activity	Comments	Selected References
Platelet-activating factor (PAF)	Newly synthesized phospholipid	Bronchoconstrictor on small airways, hypotensive, induces platelet aggregation and release of platelet 5HT. Increases vascular permeability.	Bronchoconstrictor effect partially due to TXA_2 synthesis. Released from mast cells after immunological activation.	Benveniste (1974). Stimler et al. (1983). Hamasaki et al. (1984).
Vasoactive intestinal polypeptide (VIP)	preformed peptide	Bronchodilator, inhibits mediator release, stimulates adenylate cyclase in lung.	Released from mast cells, main candidate neurotransmitter for the inhibitory innervation of the airways (non-cholinergic-non-adrenergic)	Cutz et al. (1978).
Adenosine	Preformed nucleoside	Bronchoconstrictor in asthmatics. Inhibits or stimulates mediator release depending on concentration.	Release from mast cells demonstrated. Theophylline may exert its anti-asthmatic effect by antagonism of adenosine at R_a receptors.	Sydbom and Fredholm (1982). Cushley et al. (1983). Cushley et al. (1984). Holgate et al. (1984). Marquardt et al. (1984). Hughes et al. (1984).

with histamine, acetylcholine, KCl (Orehek et al. 1975; Grodzinska et al. 1975), A23187, and antigen in sensitized tissues (Burka et al. 1981) (See Burka, 1983 for a review).

PLA_2 can be inhibited by drugs such as the anti-inflammatory glucocorticoids which induce the synthesis of a protein known as macrocortin which then causes PLA_2 inhibition (Blackwell et al. 1980; Hirata et al. 1980). Mepacrine also inhibits PLA_2 activity in airway tissue (Blackwell et al. 1978).

Following release from membrane pools, AA is rapidly enzymatically metabolized to various oxygenated products via the cyclooxygenase and/or the lipoxygenase pathways. The products of cyclooxygenation of AA include PG endoperoxides, PGE_2, PGD_2, $PGF_2\alpha$, PGI_2 and its breakdown product 6-keto $PGF_1\alpha$, and TXA_2 and its breakdown product TXB_2 (Fig. 1). PG synthesis has been demonstrated in guinea pig, human, and dog airways. The principal PG in guinea pig and dog trachea is PGE_2 (Orehek et al. 1975; Yamaguchi et al. 1976; Burka et al. 1981). The principal cyclooxygenase metabolites in lung tissue are TXA_2, PGD_2, and PGI_2 (Schulman et al. 1981). The majority of PGD_2 is probably produced by lung mast cells upon immunological activation (Peters et al. 1983), whereas the other metabolites may be produced indirectly by the action of other anaphylactic mediators upon other cells in the lung.

Selective inhibition of the cyclooxygenase enzyme can be brought about by aspirin (Vane, 1971), which irreversibly inhibits the enzyme

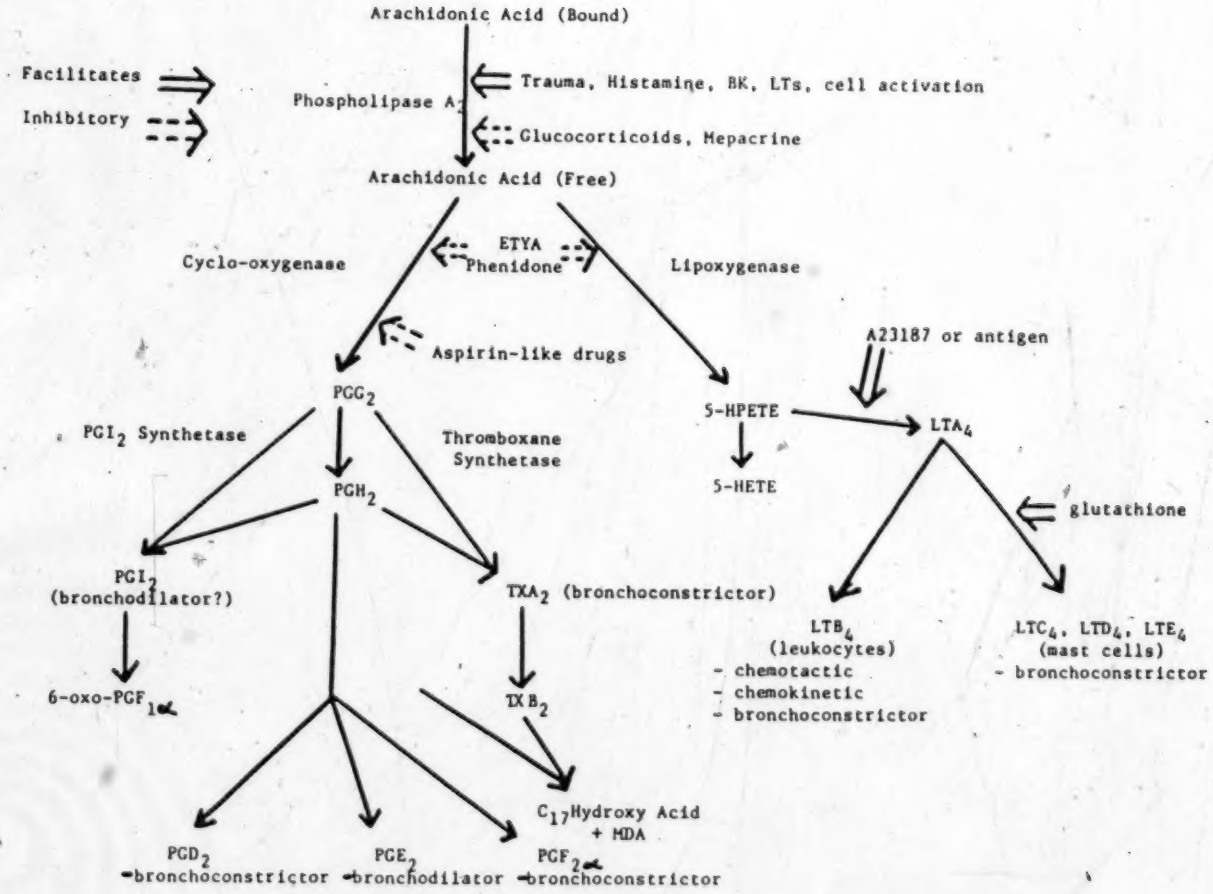


Figure 1: ARACHIDONIC ACID METABOLISM

and indomethacin, which reversibly inhibits the enzyme in guinea pig airways (Burka & Paterson, 1980) and other tissues. One possible consequence of cyclooxygenase-enzyme inhibition is diversion of AA metabolism into the lipoxygenase pathway with increases in the amount of products formed via this pathway (Engineer et al. 1978; Adcock & Garland, 1980; Burka & Paterson, 1980). Another consequence of inhibition of PG synthesis is the removal of any modulatory effects. This is described below.

1.2.1.3.2 Effects of PGs on the airways:

In 1974, Farmer et al. showed that indomethacin treatment reduced tone in guinea-pig trachea. Inhibition of PG synthesis by aspirin in guinea-pig trachea decreases the responses to low concentrations of bronchoconstrictor agents, but enhances responses to higher concentrations of bronchoconstrictors (Orehek et al. 1975; Grodzinska et al. 1975). PGE_2 levels were reduced concomitant with the above effects, PGE_2 is a weak bronchodilator in the airways and inhibition of its synthesis would lead to removal of its negative modulatory influence on the airways. Furthermore, the guinea-pig trachea can produce PGE_2 in greater amounts than $PGF_2\alpha$, 6-Keto $PGF_1\alpha$, or TXB_2 (Burka et al. 1981; Brink et al. 1981). These products may serve to regulate tone in guinea-pig trachea. PGs also have direct contractile and relaxant effects in the airways. Ghelani et al. (1980) and Chand & De Roth (1979) showed that $PGF_2\alpha$ contracts human and guinea-pig lung

parenchymal preparations. Furthermore, PGD_2 and TXA_2 can contract, and PGE_2 and PGI_2 can relax, human airways in vitro (Hutas et al. 1981) and in vivo (Walters et al. 1982; Hardy et al. 1985). Gardiner & Collier (1980) suggested the existence of contractile, relaxant, and irritant (cough reflex) receptors for PGs based on the rank order of potency of a range of PGs and analogues in mediating the above effects.

Schneider & Drazen (1980) used the 9α, 11α, (U-44069), and the 11α, 9α-epoxymethanoendoperoxide analogues to demonstrate contractile effects on guinea-pig lung and trachea. These analogues exceeded the ability of $PGF_2\alpha$, PGD_2, and histamine to constrict lung parenchymal strips, but did not exceed the ability of histamine to contract tracheal spirals. The effects of U-44069 are similar to those of TXA_2 and it has been used in place of TXA_2 as a potent constrictor of many smooth muscles including airways (Wasserman, 1976) and is more potent on smaller airways. Furthermore, it also aggregates platelets (Menzel et al. 1976).

1.2.1.4 Pharmacology of the leukotrienes

In 1938, Feldberg & Kellaway stimulated lung tissue with cobra venom and caused the release of a substance that produced characteristic slow contractions of guinea-pig intestine. Subsequent work by Kellaway & Trethewie (1940) showed that a similar factor was released from guinea-pig lung undergoing anaphylaxis and they named it "slow

reacting substance." During the 1950s, Brocklehurst coined the term slow reacting substance of anaphylaxis (SRS-A). Subsequent work (reviewed by Bach, 1982) by many groups of researchers throughout the world on the structure, origin, and functions of SRS-A in physiological and pathological states has led to the present state of knowledge on this important group of AA metabolites.

The major highlights of knowledge on SRS-A were the facts that SRS-A constituted a mixture of LTC_4, LTD_4, and LTE_4, that these were products of AA metabolism by the lipoxygenase pathway, and that these potent mediators play an important role in the pathogenesis of inflammatory and allergic states such as asthma. Many reviews have been published on various aspects of LT research. These include reviews on LT chemistry and nomenclature (Samuelsson, 1981; Corey, 1982; Hammarstrom, 1983), biological sources and release of LTs (Burka, 1981; Bach, 1982; Taylor & Morris, 1983). Biological actions of LTs and modulation of these effects (Sirois & Borgeat, 1980; Hedqvist et al. 1980; Borgeat & Sirois, 1981; Burka, 1981; Goetzl, 1981; Lewis & Austen, 1981; O'Flaherty, 1982; Bray, 1983; Piper, 1983). The above references also detail the role of LTs in asthma and other disease states.

1.2.1.4.1 Leukotriene biosynthesis:

Lipoxygenase enzymes are widely distributed in the plant and animal kingdom. AA can be oxidized by 5-, 8-, 9-, 11-, and 15-

lipoxygenases (see Burka, 1981 for review) forming 5-HPETE, 8-HPETE, 9-HPETE, 11-HPETE, and 15-HPETE, which after reduction, form the corresponding HETE, respectively. The most widely studied is 5-lipoxygenase which is found in mast cells, basophils, macrophages, and lung tissue of various species.

The production of the more polar leukotrienes occurs when 5-HPETE loses water to form an unstable epoxide, 5(6)-oxido-7,9,11,14-eicosatetraenoic acid (LTA_4) (Fig. 2). After enzymatic hydrolysis of LTA_4, polar compounds such as 5,12-diHETE (LTB_4) and its geometric isomers (Borgeat & Samuelsson, 1979a,b,c,d; reviewed by Sirois & Borgeat, 1980; Bray, 1983) are produced in eosinophils, neutrophils and mast cells. LTB_4 is a potent chemotactic and chemokinetic agent. The smooth muscle contracting LTC_4, LTD_4, and LTE_4 are also produced from LTA_4 in mast cells, macrophages, basophils and lung tissue. 5-hydroxy-6-S-glutathionyl-7,9-trans-11,14,cis-eicosatetraenoic acid (LTC_4) (Hammarstrom et al. 1979; Murphy et al. 1979) is formed after reaction of glutathione with LTA_4, catalyzed by glutathione-S-transferase. LTC_4 is further metabolized by the enzyme γ-glutamyl-transpeptidase to 5-hydroxy-6-S-cysteinylglycyl-7,9-trans-11, 14-cis-eicosatetraenoic acid (LTD_4) (Anderson et al. 1982; Morris et al. 1982). Another cysteine-containing LT, 5-hydroxy-6-S-cysteinyl-7, 9-trans-11,14-cis-eicosatetraenoic acid (LTE_4), is formed from LTD_4 by the actions of aminopeptidases inhibited by L-cysteine and other thiols (Sok et al. 1980). The structure of LTC_4 was first determined

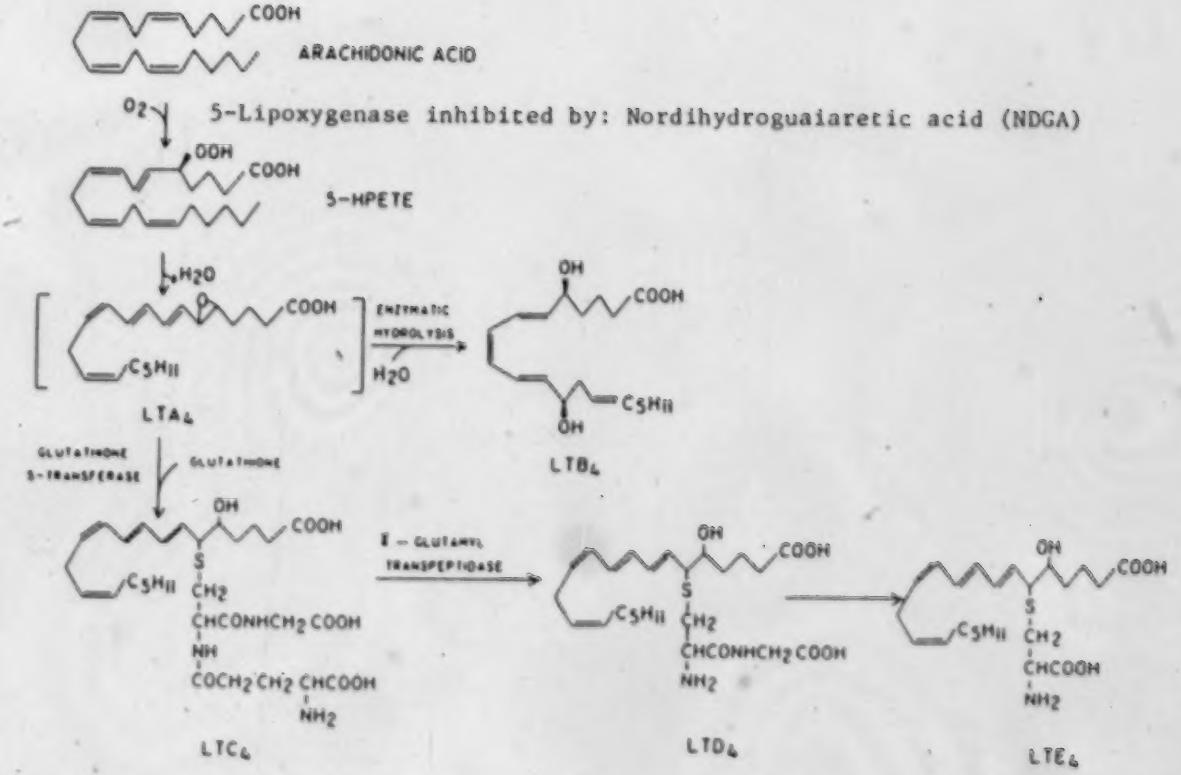


Fig. 2. Arachidonic acid metabolism by 5-lipoxygenase.

from mass spectrometry of biologically produced material from rat basophilic leukaemia (RBL) cells challenged with A23187 (Morris et al. 1980a). Further work indicated that LTs from guinea-pig lung had identical structures (Morris et al. 1980b).

The 5-lipoxygenase enzyme is calcium dependent (Jakschik & Lee, 1980; Furukawa et al. 1984). The enzyme from RBL cells has been purified using ATP linked to sepharose (Furukawa et al. 1984). These authors demonstrated that ATP stimulated enzyme activity in the presence of calcium. The 5-lipoxygenase, γ-glutamyl transpeptidase, and aminopeptidase of RBL cells were isolated by Kuo et al. (1984) who showed that the lipoxygenase enzyme is present in a soluble fraction of RBL cell homogenates whereas the latter two enzymes were membrane bound particulate enzymes. This confirmed earlier work by Jakschik et al. (1982) and Morris et al. (1982) who also studied the presence of these enzymes in guinea-pig lung and ileum.

1.2.1.4.2 Inhibition of lipoxygenase product synthesis and effects:

The pharmacological manipulation of 5-lipoxygenation of AA and subsequent enzymatic steps has received intense attention. Compounds that selectively inhibit 5-lipoxygenase have been developed and used to determine the involvement of 5-lipoxygenase metabolites in various tissue responses (Falkenhein et al. 1980).

The compounds, 1-phenyl-3-pyrazolidone (phenidone) (Blackwell & Flower, 1978), nordihydroguaiaretic acid (NDGA) (Morris et al. 1979),

and U-60,257 (piriprost) (Bach et al. 1982) have been shown to inhibit 5-lipoxygenase activity in various tissues and cells including guinea-pig lung and mast cells. As a consequence of inhibition of enzyme activity, these compounds reduce the production of SRS-A and other lipoxygenase products. Phenidone also inhibits the cyclooxygenase enzyme.

U-60,257 has been reported (Bach et al. 1982) to inhibit glutathione S-transferase activity in addition to 5-lipoxygenase. Furthermore, this compound may also antagonize LTs at their receptor sites, an action similar to that of the selective SRS-A antagonist FPL55712 (Augstein et al. 1973).

Finally, L-serine-borate complex has been shown to be an effective inhibitor of γ-glutamyl transpeptidase (Tate & Meister, 1978), hence reducing the formation of LTD_4 from LTC_4.

1.2.1.4.3 Biological sources of lipoxygenase products:

A variety of cells and tissues have been shown to produce lipoxygenase products after stimulation with antigen, A23187, AA, or other stimulants. Representative sources are summarized in Table 2. In this regards, it is noteworthy that such techniques as reverse-phase high performance liquid chromatography (RP-HPLC) (Morris et al. 1978; Borgeat & Samuelsson, 1979a,b,c; Blackwell et al. 1980), ultraviolet spectrometrey (Morris et al. 1978), bioassay on guinea-pig ileum and lung parenchymal strips, mass spectrometry (Morris et al. 1980a),

TABLE 2. Representative sources of lipoxygenase products.

Tissue or cell type	Species	Stimuli	Main Products	Selected References
Lung	Pig, human, monkey, guinea pig	A23187, antigen, AA, C5a	Histamine, LTC_4, LTD_4 LTB_4, HETE, TxA_2, PGI_2	Adams & Lichtenstein (1979). Paterson et al. (1981). Weichmann et al. (1982). Fleisch et al. (1982). Dahlen et al. (1983).
Central nervous system	Rat	A23187, AA	LTC_4, LTD_4, LTE_4	Lindgren et al. (1984).
Leukocytes	Human, horse	A23187, AA	LTC_4, LTD_4, LTB_4, 15-HETE, 5-HETE	Borgeat & Samuelsson (1979). Jorg et al. (1982). Turk et al. (1982). Weller et al. (1983). Fruteau de Laclos et al. (1984).
Mastocytoma cells	Mouse, dog	A23187, antigen	LTB_4, LTB_5, LTC_4, LTC_5 LTD_4, 5-HETE, 12-HETE, 15-HETE	Murphy et al. (1981). Phillips et al. (1983).
Macrophages (alveolar)	Rabbit, rat	A23187	LTC_4	Cade et al. (1981). Rankin et al. (1982).

and radioimmunoassay (RIA) (Levine et al. 1981), have contributed to the identification of these metabolites in various systems.

The Schultz-Dale reaction that occurs in various smooth muscle preparations (reviewed by Chand & Eyre, 1978) upon the addition of minute amounts of antigen, is due to the release of pharmacological mediators of the immediate (type I) hypersensitivity reaction. Immunologically produced lipoxygenase products from airway smooth muscle have been observed since 1940. However, the chemical nature of the mediator released from guinea-pig trachea was not fully characterized.

Many lines of evidence support the contention that the mediators released from this tissue are derived via the lipoxygenase pathway. Adams & Lichtenstein (1979), Mitchell & Denborough (1979), and Burka & Paterson (1980; 1981) demonstrated that antigen-induced contractions of guinea-pig tracheal spirals were enhanced by indomethacin pretreatment, and that antihistamines reduced the early phase (0-10 minutes) of the contractile response. By contrast, lipoxygenase inhibitors and the selective SRS-A antagonist FPL55712, reduced the late phase (15-60 minutes) of the contractile response. These observations agreed with the hypothesis that histamine and SRS-A contribute to the early and late phase of antigen-induced contractions, respectively. Furthermore, indomethacin-pretreatment enhances contractions by removing the modulatory influence of relaxant PGs on the trachea (Orehek et al. 1975) and possibly cause the diversion of AA into the lipoxygenase

pathway, hence producing more lipoxygenase metabolites, leading to an enhanced response (Burka & Paterson, 1980; Adcock & Garland, 1980). Finally, Yen & Kreutner (1980), Mitchell & Denborough (1980), and Burka & Paterson (1980) showed that AA relaxed guinea-pig trachea and contracted lung parenchymal strips. However, after indomethacin pretreatment, AA-induced relaxations of the trachea were converted to contractions with a concomitant reduction in PGE_2 synthesis (Burka & Paterson, 1980), and contractions of parenchymal strips were reduced with a concomitant reduction in TXA_2 synthesis (Mitchell & Denborough, 1980). Furthermore, lipoxygenase inhibitors and FPL55712 reduced the AA-induced contractions of trachea after indomethacin-pretreatment. Hence, the possibility that LTs may be synthetized in guinea-pig trachea after antigen, A23187, and AA challenge. This possibility and the role of calcium in the above processes was investigated in this thesis.

1.2.1.4.4. Effects of leukotrienes on the airways:

LTC_4, LTD_4, and LTE_4 are 100-1,000 times more potent than histamine in contracting airway smooth muscle from man, guinea pig, and monkey. Furthermore, the LTs are more potent on the small terminal airways, e.g., bronchioles, than the large conducting airways e.g., trachea. LT effects, in vitro, develop slowly and are long lasting despite exhaustive washing of airway tissues. (Hedqvist et al. 1980; Drazen et al. 1980; Hanna et al. 1981; Sirois et al. 1981;

Piper & Samhoun, 1981). Also, LTB_4 contracts guinea-pig lung parenchymal strips and to a lesser extent guinea-pig tracheal spirals (Sirois et al. 1981). LTB_4 does not contract guinea-pig ileum, whereas LTs C_4, D_4 and E_4 do; LTD_4 is the most potent.

Administration of LTC_4, LTD_4, and LTE_4 to guinea pigs in vivo results in a long lasting increase in airway resistance and a decrease in lung compliance. The latter persisted after the former returned to normal baseline levels (Drazen, 1980; Drazen et al. 1982). In addition to the above effects, LTC_4 and LTD_4 induce vasoconstriction and increased vasopermeability, respectively (Drazen et al. 1980; Drazen et al. 1982).

In vitro human bronchial smooth muscle contracts to both LTC_4 and LTD_4 (Jones et al. 1982). This effect is not blocked after atropine, mepyramine, or indomethacin-pretreatment. However, responses to both LTs are reduced by FPL55712. Human asthmatic lung strips also contract to LTs, but there is no hyperreactivity to LTs when compared to control lungs (Schellenberg & Foster, 1984). These findings are supported by those of Griffin et al. (1983), who showed that asthmatics are not hyperreactive to the bronchoconstrictor effects of LTD_4.

LTC_4-, LTD_4-, and LTB_4-induced contractions of guinea-pig lung parenchymal strips are reduced by indomethacin-pretreatment (Piper & Samhoun, 1981 and 1982; Creese et al. 1981; Sirois et al. 1982). The effects of LTB_4 on the same tissue are reduced by inhibition of

cyclooxygenase or by inhibition of PLA_2 with mepacrine (Sirois et al. 1982). The reduction of LT-induced contractions of lung parenchyma by cyclooxygenase blockade, occurs with a concomitant decrease in TXA_2 synthesis. Also, others have reported the synthesis of the bronchodilator PGI_2 after LT-induced contractions of guinea-pig lung strips (Creese et al. 1981; Omini et al. 1981). Hence, on the guinea-pig lung parenchyma, LT effects are mediated partly via the synthesis of cyclooxygenase products, such as the bronchoconstrictor TXA_2. However, in human isolated bronchial and lung parenchymal strips, LTB_4-, LTC_4-, and LTD_4-induced contractions were not affected by cyclooxygenase inhibition (Samhoun & Piper, 1984).

1.2.1.4.5 Effects of leukotrienes on the central nervous system:

The effects of the LTs on CNS function have not been widely studied. However, the following studies suggest a possible role for lipoxygenase metabolites in the CNS.

Palmer et al. (1980) and (1981) showed that pressure injection of LTC_4 and LTD_4 onto rat cerebellar Purkinje fibers caused a prolonged excitatory action; i.e., increase in firing rate, which could be antagonized by FPL55712. LTB_4 had no effect in the above system. Recently, LTs have been shown to be synthesized in various areas of rat brain including cerebellum (Lindgren et al. 1984). Finally, LTC_4 in low concentrations has been shown to stimulate luteinizing hormone release from rat pituitary cells in culture (Hulting et al. 1985).

1.2.1.4.6 Leukotriene receptors:

The greater potency of LTD_4 relative to LTC_4 in contracting guinea-pig lung parenchymal strips and tracheal spirals, and the greater antagonistic effect of FPL55712 on LTD_4-induced contractions suggests that LTC_4 and LTD_4 probably act at different receptor sites (Drazen et al. 1980; Krell et al. 1981; Snyder & Krell, 1984). Moreover, Fleisch et al. (1982) showed that FPL55712 was more potent in antagonizing LTD_4-induced contractions of ileum than those induced on isolated airways of guinea pig. This suggests that LTD_4 receptors may be different in various smooth muscles.

The fact that LTC_4 can be converted to LTD_4 which can be further converted to LTE_4 by enzymes present in airway smooth muscle and in guinea-pig ileum (Morris et al. 1982) (see Fig. 2) complicates the study of receptor subtyping in these tissues. However, by the judicious use of inhibitors of γ-glutamyl transpeptidase (inhibitor: serine borate complex) and aminopeptidase (inhibitor: cysteine) the relatively undistorted effects of LTC_4 and LTD_4 can be assessed. Snyder et al. (1984) demonstrated that under conditions where LTC_4 and LTD_4 remain unmetabolized their potency increased since conversion to LTE_4 was prevented. Furthermore, the slight antagonistic effects of FPL55712 on guinea-pig trachea against LTC_4 were abolished in the presence of serine borate complex (Snyder & Krell, 1984). These findings suggest that LTC_4 acts at a different receptor site from LTD_4.

Radioligand binding studies of the LTs has confirmed the previous pharmacological evidence. LT-binding sites equated with receptors based on their high affinity, pharmacological specificity, tissue specificity, saturability, reversibility, and stereoselectivity have been demonstrated for LTC_4 and LTD_4 in membranes of guinea-pig lung (Pong & DeHaven, 1983; Bruns et al. 1983; Mong et al. 1984 a,b; Cheng & Townley, 1984 a,b,c). Receptor sites for LTE_4 appear to be identical to those for LTD_4 (Cheng & Townley, 1984c). Specific LTC_4-binding sites have been shown in rat lung (Pong et al. 1983), a smooth muscle cell line derived from hamster vas deferens (Krilis et al. 1983; Clark et al. 1984), human fetal lung (Lewis et al. 1984), guinea-pig ileum (Krilis et al. 1984; Nicosia et al. 1984), guinea-pig uterus (Levinson, 1984), and guinea-pig brain (Cheng et al. 1985).

The order of potency for inhibition of $[^3H]LTD_4$ binding in guinea-pig lung is $LTD_4 > LTE_4 > LTC_4 >> FPL55712$ (Pong & DeHaven, 1983; Cheng et al. 1985). In the same tissue, the dissociation constant (K_d) for LTD_4 binding at saturation and at 20°C ranges from 0.5 nM to 10 nM in various studies. The number of binding sites at saturated equilibrium (B_{max}) range from 384 to 2100 fmol/mg protein. Some tissues do not exhibit specific LTD_4 binding sites and LTD_4 can compete with $[^3H]LTC_4$ in binding to the LTC_4 receptors with an inhibitory constant (K_i) supportive of the presence of two receptor sites for the LTs. Furthermore, FPL55712 can inhibit LTD_4 binding with K_i values similar to K_b values obtained in

pharmacological studies (Pong & DeHaven, 1983). FPL55712 does not inhibit LTC_4 binding effectively.

LTC_4 may be metabolized to LTD_4 during the binding assay and may give rise to curvilinear Scatchard plots and erroneous competition data. The inclusion of serine borate complex (45 mM) in the assay minimizes possible degradation of the radioligand and changes the order of potency for inhibition of $[^3H]LTC_4$ binding in guinea-pig lung from $LTC_4 = LTD_4 > LTE_4$ to $LTC_4 >> LTD_4 >> LTE_4$ and for inhibition of $[^3H]LTD_4$ binding from $LTC_4 = LTD_4 > LTE_4$ to $LTD_4 > LTE_4 = LTC_4$ (Cheng & Townley, 1984b).

LTC_4 binding is not regulated by guanine nucleotides or monovalent cations but is divalent cation sensitive (Pong et al. 1983 and others). LTD_4 binding is reduced by monovalent cations especially Na^+. A 50% reduction in the B_{max} by Na^+ (50 mM) was observed by Mong et al. 1985. Na^+ does not affect the K_D. Mg^{2+}, Mn^{2+}, and Ca^{2+} at 5-10 mM increase the B_{max} of LTC_4 and LTD_4 receptors in various tissues to 175-200% of control levels without affecting the K_D (Pong & DeHaven, 1983, Bruns et al. 1983; Mong et al. 1985). Addition of GTP increases the rate of dissociation of bound LTD_4 (Pong & DeHaven, 1983). Furthermore, guanine nucleotides reduce the level of assayable high affinity LTD_4 binding sites in lung (Pong & DeHaven, 1983; Bruns et al. 1983; Mong et al. 1985). Guanine nucleotides (the nonhydrolysable analogs, GTPγS and GppNHp, being most potent) reduce the B_{max} but do not affect the K_D of the residual binding, possibly they

shift LTD_4 receptors into a lower affinity and less easily assayable form (Mong et al. 1985).

1.2.1.4.7 Possible intracellular mechanism of action of LTD_4:

The inhibition of LTD_4 binding to its receptors by Na^+ and guanine nucleotides indicates that the receptor may be linked to adenylate cyclase in an inhibitory manner. This is because similar guanine nucleotide and Na^+-dependent regulation of other receptors that inhibit adenylate cyclase has been observed. These receptors include muscarinic receptors in mouse brain, rat brain and ileum (Sokolovsky et al. 1980; Ehlert et al. 1980; Olianas et al. 1982), adenosine R_i receptors in brain and fat cells (Cooper et al. 1980; Gavish et al. 1982; Ukena et al. 1984), opiate receptors in brain and ileum (Koski et al. 1982), α_2-adrenergic receptors in platelets (Tsai et al. 1979; Hoffman et al. 1980; Motulsky et al. 1983), and GABA receptors in rat cerebellum (Hill & Bowery, 1981; Wojick & Neff, 1983).

The effects of guanine nucleotides are transmitted to the receptor via the GTP regulatory proteins of adenylate cyclase (Rodbell, 1980; Jakobs et al. 1981). These hormones have all been shown to inhibit adenylate cyclase (for reviews see Jakobs et al. 1981; Cooper, 1982; Jakobs et al. 1984) and section 1.2.3.1.1.

By analogy with the above hormone-receptor systems, it seems likely that LTD_4 may interact with its receptor in a similar fashion.

Further support for this hypothesis was the observation that LT-induced contractions of guinea-pig tracheal smooth muscle occurred along with a reduction in cyclic AMP levels. FPL 55712 blocked the contractile response, and the reduction in cyclic AMP (Hedman & Anderson, 1982). Since LTs exert effects on airway smooth muscle and on rat cerebellum, these tissues were studied in this thesis with regards a possible inhibitory action of LTD_4 on adenylate cyclase.

1.2.2. Autonomic control of the airways

Autonomic nervous activity modulates a variety of body functions. The airways are no exception. Reviews on the relative contributions of sympathetic, parasympathetic, and other inhibitory pathways to airway smooth muscle (ASM) include those by Richardson (1979) and Nadel & Barnes (1984). Table 3 briefly summarizes the various pathways exerting influences on ASM.

1.2.2.1 Parasympathetic and sympathetic nervous system:

Radioligand binding studies and autoradiographic localization studies demonstrate the presence of muscarinic receptors, (Murlas et al. 1982; Barnes et al. 1983), β, and α receptors (Barnes et al. 1982; Nadel & Barnes, 1984) in the airways of various species. The β_2 receptor subtype predominates over the β_1 receptor in the airways (β_2: β_1, 85:15) (Nadel & Barnes, 1984).

The effects of muscarinic and α- and β-adrenergic receptor

TABLE 3. Neurohumoral influences acting on airway smooth muscle (ASM).

System	Postganglionic Transmitter	Effect on airways	Degree of innervation	Pharmacologic receptors present
Cholinergic (vagus nerve)	Acetylcholine	Contraction of ASM. Stimulation of mucus secretion	First 6 generations of airways innervated. Small airways not innervated.	Muscarinic receptors blocked by atropine. Decreasing density with decreasing airway diameter.
Sympathetic α_1 and α_2	Norepinephrine	Contraction. Secretion of mucus.	No major innervation of ASM in man, guinea pig, dog, monkey, and rat	α_1 and α_2 receptors present throughout Effective during β blockade
β_2	Circulating epinephrine	Bronchodilation. Inhibition of mediator release. Slight stimulation of mucus release.	No major innervation (as above)	β_2 receptors
Nonadrenergic noncholinergic (NANC)	Possibly vasoactive intestinal polypeptide (VIP)	Bronchodilation. Inhibition of mediator release. Inhibition of mucus release.	Innervation present	VIP receptors documented in lung of variety of species.

stimulation on airway functions are shown in Table 3. The effects of β-adrenoceptor stimulation may be related to increases in intracellular levels of cyclic AMP (Sullivan & Parker, 1976; Lefkowitz et al. 1983).

1.2.2.2 Non-adrenergic non-cholinergic (NANC) inhibitory innervation:

NANC inhibitory innervation has been documented in a variety of smooth muscles (Burnstock, 1972). The neurotransmitter may be vasoactive intestinal peptide (VIP) (Richardson, 1981; Diamond & Richardson, 1982; Said, 1982). Other possible candidate neurotransmitters include purines (Burnstock, 1972), substance P, enkephalins, prostaglandins, and a host of others (See Richardson, 1981; Diamond & Richardson, 1982).

NANC innervation has been demonstrated to exert substantial influence in human (Richardson & Beland, 1976) and in guinea-pig airways (Yip et al. 1981; Grundstrom et al. 1981). In fact, it has been estimated that 60% of nerve-induced inhibition in man and guinea pig is due to NANC activation (Richardson, 1981; Richardson & Beland; 1976; Diamond & Richardson, 1982).

In the guinea pig, two lines of evidence implicate VIP as being the NANC transmitter. First, antisera to VIP reduced the relaxation obtained after stimulation of the NANC innervation, and secondly, by using radioimmunoassay, it was demonstrated that relaxation can be correlated to the levels of immunoreactive VIP released (Matsuzaki et

al. 1980). Furthermore, VIP containing neurones have been immunocytochemically localized in close vicinity to airway smooth muscle in dog, man, and guinea pig (Uddman et al. 1978; Dey & Said, 1980; Richardson, 1981).

VIP can prevent the bronchoconstriction induced by histamine, kinins and $PGF_{2}\alpha$ (Said et al. 1974). VIP receptors have been demonstrated on rat lung and activate adenylate cyclase linked to these receptors (Robberecht et al. 1981). VIP also increases cyclic AMP levels in guinea-pig trachea (Fransden et al. 1978), and inhibits antigen-induced mediator release from guinea-pig lung (Undem et al. 1983). Finally, VIP has been shown to inhibit mucus secretion by human airways in vitro (Coles et al. 1981).

The protective effects of VIP (bronchodilation, inhibition of mediator release, and inhibition of mucus secretion) on the airways serve to highlight the fact that an abnormality in NANC innervation may lead to asthmatic hyperreactivity. A substantial amount of evidence is still needed to establish VIP as the prime neurotransmitter in the NANC innervation of the airways.

1.2.3 Basic regulatory processes in the airways:

In this section, consideration is given to regulatory processes exerted upon airway smooth muscle and secretory cells in the lung by cyclic AMP and calcium. The pathogenesis of asthmatic hyperreactivity (1.3) may find correlates in changes or dysfunctions in the regulatory

roles of these secondary messenger of cellular function.

1.2.3.1 Adenylate cyclase:

Adenylate cyclase is a membrane bound enzyme universally distributed in animal cells. The enzyme catalyzes the conversion of ATP into cyclic AMP and pyrophosphate.

Various stimulatory and inhibitory hormones regulate adenylate cyclase activity in a variety of cell types (Cooper, 1982 for review). These hormones hence control the intracellular levels of cyclic AMP in the cell and thus affect the transfer of information, ultimately leading to the characteristic cellular response to the particular hormone.

The components of this information transmission system consist of the hormone receptor (R) which acts as the recognition transduction element, the guanine nucleotide binding proteins, Ns and Ni which mediate the effects of R occupation by stimulatory and inhibitory hormones, respectively, onto the catalytic subunit (C) of the enzyme (Rodbell, 1980). Activation of C involves the exchange of inactive GDP for active GTP at Ns or Ni. This exchange is probably initiated by hormone binding to R. Ns and Ni are probably two separate proteins (Hildebrandt et al. 1983). C utilizes a complex formed between Mn^{2+} or Mg^{2+} and ATP to produce cyclic AMP.

Various models of the interaction of cyclase components in stimulation and inhibition of the enyzme induced by hormones, have

been developed (for review see Muller, 1985). The "exchange-collision-coupling model" of Levitski (1982) and the "disaggregation-coupling" model of Rodbell (1980) seem to account for experimentally observed interactions between R, Ns, Ni and C. In Levitski's model and other models, GTP, at a concentration range of 0.01 - 0.1 μM for stimulation and at a concentration range of 0.2 - 6.0 μM for inhibition, is essential for stimulatory and inhibitory hormone effects on adenylate cyclase.

In both the above models, high affinity binding of a hormone to its receptor is converted to a low affinity interaction upon the binding of guanine nucleotides to Ns or Ni. Furthermore, Rodbell's model implies reciprocal effects, i.e. hormone binding facilitates the interaction of guanine nucleotides with Ns or Ni.

Studies of adenylate cyclase were greatly clarified following the discovery of forskolin (see Seamon et al. 1981). This diterpene increases cyclic AMP synthesis in all cyclase systems studied to date (reviewed by Seamon & Daly, 1981b). Forskolin does not utilize guanine nucleotides in its rapid and massive (i.e. relative to hormonal stimulants) stimulation of the enzyme (Seamon & Daly, 1981a). Furthermore, forskolin enhances the ability of stimulatory hormones to activate the enzyme. This implies an interaction of forskolin with the processes of Ns activation of C. Forskolin has been shown to bind to rat brain membranes (Seamon et al. 1984). Analogs of forskolin which do not activate adenylate cyclase did not compete for the

binding site. It is now currently accepted that forskolin activates C directly, although interactions with Ns cannot be ruled out.

1.2.3.1.1 Characteristics of adenylate cyclase inhibition:

Non-hydrolysable analogs of GTP can inhibit the basal and the forskolin-stimulated enzyme (Seamon & Daly, 1982). In fact, this effect is exploited to determine the presence of Ni in a given cell. Although Ns has been shown to exist (functionally and physically) in a variety of cells, the situation with Ni is not as clear.

The reduction in cyclic AMP levels in cells may act as a signal to promote various other cellular activatory processes including phospholipid metabolism, release of AA from membrane pools and its subsequent peroxidation into active metabolites, and Ca^{2+} entry which activates the latter processes, prior to the tissue-specific biological response (Michell & Kirk, 1980; Jakobs & Schultz, 1980; Jakobs et al. 1984; Berridge & Irvine, 1984; Baron et al. 1984).

Common features shared by hormone-induced and GTP analog-induced inhibition of cyclase include a greater effect at low temperatures (20°C vs 37°C), at low Mg^{2+} concentrations in the assay (around 1-2 mM), a higher GTP requirement than for enzyme-stimulating receptors (does not apply to GTP analogs), and sensitivity to proteases (proteases abolish cyclase inhibition). Furthermore, Mn^{2+} concentrations over 1 mM have been shown to reduce adenylate cyclase inhibition. A high Na^{+} concentration (100 mM) has been shown to optimize inhibition

induced by certain receptors e.g. the α_2 receptor in the fat cell system (Aktories et al. 1979; Aktories et al. 1981; reviewed by Jakobs et al. 1981).

Inhibitory hormones generally reduce basal adenylate cyclase activity by about 30-80%. In the presence of a stimulatory hormone or compound; e.g., forskolin, the degree of inhibition is generally greater than observed if the enzyme is in the basal state.

1.2.3.2 Cyclic AMP and the airways:

In the airways, cyclic AMP may be involved in smooth muscle contractility, mediator release, and mucus secretion. In this section some of the evidence for cyclic AMP involvement in airway smooth muscle contractility is discussed. The area has been reviewed (Baer, 1974; Andersson & Nilsson, 1977; Diamond, 1978; Hardman, 1981).

Since the original studies of Sutherland & Rall (1960), the second messenger role of cyclic AMP in cellular function has received intense attention. Hormones activating smooth muscle relaxant receptors such as the β-adrenoceptor, adenosine receptor, various relaxant PG receptors, and other hormonal receptors have been studied in airway, intestinal, vascular and uterine smooth muscle. Cyclic AMP levels in tissues can be increased or decreased by activation of the membrane bound enzyme, adenylate cyclase, or by inhibition or stimulation of cyclic nucleotide phosphodiesterase.

It has been suggested, as a general working hypothesis, by

Sutherland (1974) that β-adrenoceptor effects are mediated by increases in cellular levels of cyclic AMP. It has been theorized that decreases in cyclic AMP levels, or increases in cyclic guanosine monophosphate (cyclic GMP), will promote contraction of smooth muscle (see Baer, 1974; Diamond, 1978 and others). This simplified scheme has not found convincing evidence and the roles of cyclic AMP and cyclic GMP in smooth muscle relaxation are still controversial.

The above hypothesis is based on the fact that cyclic AMP and some of its analogues can induce smooth muscle relaxation. Secondly, adrenergically induced smooth muscle relaxation can sometimes be correlated with tissue levels of cyclic AMP. Both effects are abolished by β-adrenoceptor antagonists. Thirdly, inhibitors of cyclic AMP phosphodiesterases can induce relaxation, increase cyclic AMP levels, and potentiate effects induced by other relaxant agents. These observations are derived from studies on vascular, uterine, airway and intestinal smooth muscle.

Various authors have shown that airway smooth muscle relaxes in response to β-adrenoceptor stimulation concomitant with increases in tissue levels of cyclic AMP. These two effects generally exhibit similar concentration-response and time-response relationships and are similarly blocked by propranolol (Murad, 1974; Ohkubo et al. 1976; Katsuki & Murad, 1977; Rinard et al. 1983; Lau & Lum, 1983). Furthermore, theophylline and other phosphodiesterase inhibitors can potentiate relaxation and cyclic AMP increases following stimulation

with β agonists (Lohmann et al. 1977; Polson et al. 1982)

Other hormones studied in airway smooth muscle include PGI_2 (Macdermot & Barnes, 1980), and VIP (Robberecht et al. 1981). Both substances increase cyclic AMP levels by stimulation of adenylate cyclase in guinea-pig and rat lung, respectively. Recent studies utilizing forskolin have demonstrated that this diterpene is a potent inhibitor of mediator release from the airways. Burka (1983 a,b) has demonstrated that forskolin inhibits antigen- and calcium ionophore-induced contractions of guinea-pig airways and is a potent bronchodilator in vitro. Kreutner et al. (1984) have also shown that forskolin is a bronchodilator when administered by aerosol. It prevents and reverses allergic bronchoconstriction in guinea pigs. The initial observations of Assem & Schild (1969) that epinephrine can inhibit antigen-induced histamine release from human lung and the recent work with forskolin further demonstrate a role for cyclic AMP in mediator release (Sullivan & Parker, 1976; Tung & Lichtenstein, 1981; Alms & Bloom, 1982) and smooth muscle contractility (Scheid et al. 1978). In fact, current bronchodilator therapy in asthma probably depends on these two mechanisms of action.

1.2.3.3 Calcium and the airways:

The important roles of Ca^{2+} in the airways has been well documented (for reviews see Middleton, 1980 and 1984; Rasmussen & Barrett, 1984). Unfortunately, there are very few studies of the

molecular basis of contraction of airway smooth muscle and its Ca^{2+} regulation per se. It is only with the advent of calcium entry blockers in the therapy of asthma (Goodman, 1981; Fanta & Drazen, 1983; Triggle, 1983; Barnes, 1983) that more attention has been paid to Ca^{2+} regulation of airway smooth muscle contractility.

1.2.3.3.1 Sources of utilizable calcium in the airways:

Creese & Denborough (1981) showed that contractile responses of guinea-pig trachea to KCl, histamine, acetylcholine, and $PGF_2\alpha$ were reduced, but not abolished, in a Ca^{2+}-free medium. These authors concluded that there were two sources of Ca^{2+} in the trachea: an extracellular source probably in equilibration with the medium and an internal source of utilizable Ca^{2+}. Furthermore, responses to high concentrations of bronchoconstrictors were less dependent on extracellular Ca^{2+}. Similar results were obtained by Farley & Miles (1978) using dog tracheal smooth muscle. Burka (1984) showed that calcium-ionophore-A23187-induced contractions of guinea-pig trachea were totally dependent on extracellular Ca^{2+}, but that antigen-induced contractions were partially dependent on intracellular calcium.

1.2.3.3.2. Calcium channels in smooth muscle:

During periods of stimulation of smooth muscle by Ca^{2+} mobilizing agents, intracellular Ca^{2+} concentrations may increase up to a 1000 times those in the relaxed state (Middleton, 1984). This rise occurs

via entry of Ca^{2+} into the cell through specific conductance channels, or via mobilization of intracellular bound Ca^{2+} from sarcoplasmic or endoplasmic reticulum, mitochondria, or other Ca^{2+} sequestering organelles (Webb & Bhallau, 1976). Mechanisms also exist to return Ca^{2+} concentrations to resting levels (Rasmussen & Barrett, 1984).

Ca^{2+} movement across the membrane occurs through specific channels activated in response to depolarization (i.e. voltage-dependent channels) or in response to receptor occupation by a contractile agonist (i.e. receptor-operated channel) (see Triggle, 1983). A group of drugs known collectively as Ca^{2+} channel blockers, including verapamil, D600, nifedipine, nitrendipine, and diltiazem have a high affinity for Ca^{2+} channels in a variety of cells including airway smooth muscle cells. These compounds can selectively inhibit the activation of Ca^{2+} channels especially the voltage-dependent channels and inhibit Ca^{2+}-mediated effects (Triggle, 1981, Goodman, 1981; Triggle, 1983; Barnes, 1983).

An intracellular Ca^{2+} antagonist, 8-(diethylamino)octyl-3,4,5-trimethoxybenzoate hydrochloride (TMB-8) (Chiou & Malagodi, 1975) stabilizes intracellular Ca^{2+} ions to binding sites and inhibits contractions of skeletal and smooth muscle.

1.2.3.3.3 Excitation-contraction coupling in smooth muscle:

A rise in intracellular Ca^{2+} concentrations activates the calcium binding protein, calmodulin (Cheung, 1980; Brostom & Wolff, 1981).

The Ca^{2+}-calmodulin complex interacts with inactive myosin light chain kinase, which becomes activated and subsequently phosphorylates myosin to myosin-PO_4, which in the presence of the contractile protein, actin, activates the myosin ATPase. Subsequent cleavage of ATP yields energy which is utilized in muscle shortening according to the sliding filament theory (See Stull & Sanford, 1981; Middleton, 1984; Rasmussen and Barrett, 1984).

Sparrow et al. (1984) recently demonstrated that calmodulin increased sensitivity to Ca^{2+} in skinned muscle fibers from guinea-pig trachealis. Furthermore, a cyclic AMP-dependent protein kinase inhibited tension development induced by the Ca^{2+}-calmodulin complex after β-adrenergic stimulation. An earlier study by Hogaboom et al. (1982) using tracheal smooth muscle demonstrated cyclic AMP- and cyclic GMP-dependent phosphorylation of 2 membrane proteins ultimately involved in transport of Ca^{2+} into microsomes via phosphorylation of a Ca^{2+}-transport ATPase.

1.2.3.3.4 Calcium antagonists and asthma:

Calcium entry blockers have demonstrable therapeutic use in certain types of asthma (Goodman, 1981; Barnes, 1983; Fanta & Drazen, 1983; Middleton, 1984). Patel (1981a) showed that nifedipine prevented exercise-induced asthma in a group of fifteen asthmatics. Other authors have demonstrated similar effects of nifedipine, verapamil, and cinnarizine against exercise-induced and cold

air-induced bronchoconstriction in asthmatics (see Middleton, 1984). Furthermore, Patel (1981b) showed that verapamil given by inhalation did not alter histamine- and methacholine-induced bronchoconstriction in asthmatics. Similar results were obtained by McIntyre et al. (1983). They concluded that calcium antagonists inhibit mediator release and not the smooth muscle bronchoconstriction effected by asthmatic mediators. This conclusion was partly supported by the findings of Henderson et al. (1983) who showed that in vitro contraction of sensitized guinea-pig trachea and human bronchial muscle induced by acetylcholine, histamine, and specific antigen were either unaffected by nifedipine (100 μM) or reduced by about 50% in human bronchial muscle. Moreover, these authors showed that nifedipine-pretreatment inhibited antigen-induced bronchoconstriction in asthmatics challenged with grass pollen. These findings contradict those of So et al. (1982) who showed that inhaled verapamil and nifedipine did not alter antigen-induced bronchoconstriction in eight asthmatics, and Patel et al. (1983) who also showed that inhaled verapamil did not affect allergen-induced bronchoconstriction in human asthmatics.

Weiss et al. (1982) showed that in vitro verapamil inhibited antigen-induced contractions of guinea-pig tracheal spirals. However, antigen-induced histamine release was unaffected. Fanta et al. (1982) also showed that nifedipine reversed existing tone in guinea-pig tracheal spirals and inhibited constriction induced by histamine and

carbachol in tracheal and parenchymal preparations in vitro.

The above representative studies leave no doubt that calcium-entry blockers are effective in preventing or reversing asthmatic bronchoconstriction. However, there is controversy surrounding their mechanisms of action. They may work by inhibition of mediator release, by inhibition of smooth muscle contraction, or both.

1.3 STUDIES ON MODELS OF ASTHMA: MECHANISMS OF AIRWAY HYPERREACTIVITY

Unfortunately, most animal models of human diseases are not "true" models of the human situation. This is basically the situation with animal models of human bronchial asthma. Models have included dog, guinea pig, monkey, sheep, and rats, and airway tissues and cells derived from these models, and from human sources. Although a huge amount of data has accumulated from studies, as yet there is no concrete theory to describe the origin and pathogenesis of human asthmatic hyperrereactivity. However, a lot of knowledge has been gained on processes of anaphylactic mediator release, and the effects of mediators that may likely translate into the pathologic induction of hyperreactivity. In this section various studies of animal models and their human counterpart will be reviewed in order to demonstrate the variety of hypotheses that have been originated to account for airway hyperreactivity.

1.3.1 Sensitization-dependent changes in pharmacologic receptors:

Szentivanyi (1968) first proposed that β-receptors are hypoactive in the airways of asthmatics. Hence, the relaxant influence these receptors mediate is lost and bronchoconstrictor tone overrides the fine balance of airway caliber leading to an enhanced broncho-constrictor response to various stimulants. These observations were supported by the fact that propranolol induces bronchoconstriction in asthmatics, but not in normal individuals (Richardson & Sterling, 1969). Szentivanyi's theory has gained a lot of support especially since it has been reported that a variety of β-mediated effects such as vasodilation, hyperglycemia, platelet aggregation, increases in urinary cAMP, and increases in blood lactate were decreased in asthmatics (Sly, 1981).

An extension of the β-hyporesponsiveness theory of asthma is the premise that excitatory α-adrenoceptor and muscarinic receptor function is increased in asthmatic individuals. This was based partly on the fact that α-adrenergic antagonists and atropine can prevent exercise- and histamine-induced bronchoconstriction in asthmatics (Bianco et al. 1974; Kerr et al. 1970). Furthermore, Kaliner et al. (1982) have shown that allergic subjects, including asthmatics, have muscarinic-receptor, α-adrenoceptor hyperresponsiveness, and β-adrenoceptor hyporesponsiveness (Shelhamer et al. 1983).

In opposition to the hypoactive beta-adrenoceptor theory of asthma, it must be noted that most asthmatic subjects have a history

of treatment with β-adrenergic agonists, and the observed β-hyporesponsiveness may be a manifestation of receptor desensitization and/or down regulation of receptors (Lefkowitz et al. 1983). Furthermore, the stress of asthma may cause metabolic changes leading to the observed effects, i.e., receptor hyporesponsiveness is a consequence rather than a cause of asthma.

Radioligand binding studies have demonstrated significantly lower β-receptor density in mononuclear leukocytes of asthmatics not taking any medication, whereas in asthmatics on medication, including β-agonists, there was a significantly reduced β-adrenoceptor binding density in mixed leukocytes, mononuclear cells and in polymorphonuclear leukocytes (Sano et al. 1983). Studies by Makino et al. (1983) have demonstrated diminished cyclic AMP responses of asthmatic lymphocytes after stimulation with β-agonists. Furthermore, there was a significant correlation between β-hyporesponsiveness and muscarinic-hyperresponsiveness determined by measurement of the respiratory threshold to acetylcholine. However, another study (Meurs et al. 1982) found no difference between asthmatic and normal lymphocytes in terms of β-adrenoceptor binding density and the cyclic AMP response to β-agonists.

Animal studies have produced conflicting evidence. Barnes et al. (1980) showed that in the ovalbumin-sensitized and challenged guinea-pig model of asthma there was a decreased pulmonary β-receptor density and an increased α-receptor density. Furthermore, the

magnitude of adenylate cyclase activation by isoproterenol in lungs from these animals was less than that of control animals. The animal model used by Barnes et al. (1980) exhibited symptoms of asthma; i.e., coughing, wheezing, and hyperreactivity to bronchoconstrictors. Therefore, Cheng & Townley (1982) investigated the effects of bronchoconstriction on β-adrenoceptor density and showed that only in animals that were exposed to bronchoconstrictors like histamine was a reduced binding density observed. Therefore, they concluded that β-adrenoceptor hypoactivity can result as a consequences of the stress induced by bronchoconstriction.

Studies comparing naturally ascaris-sensitive beagle dogs and normal beagle dogs demonstrated that α-adrenoceptor-mediated bronchoconstriction only occurred in the allergic dogs (Malo & Wasserman, 1983). Furthermore, this effect was potentiated by propanolol/indomethacin pretreatment. Hence, it appears that α-adrenoceptor-induced bronchospasm only plays a role in allergic dogs and not in the unsensitized controls. Finally, Bongrani et al. (1983) showed that β-adrenoceptor blockade potentiated bronchospasm induced by acetylcholine, histamine, or LTC_4.

The above representative studies demonstrate the diversity of results obtained from investigations of β-adrenoceptor function in asthma. There is clearly a need to investigate the role of other relaxant receptors, e.g., the VIP receptor, in the airways. Furthermore, it is apparent that a variety of factors, including prior

administration of β-agonists and stresses of bronchoconstriction, can contribute to the lack of uniformity of the conclusions reached.

1.3.2 Sensitization-dependent changes in cyclic AMP and PGs:

Various studies have suggested alterations in cyclic AMP levels after immunological sensitization. Sydbom et al. (1979) showed that the basal levels of cyclic AMP in rat mast cells was significantly reduced by sensitization, and that anaphylactic histamine release was increased. Cyclic GMP levels were unchanged by sensitization and the cyclic AMP/cyclic GMP ratio was decreased. However, Mathe et al. (1978) demonstrated earlier a decreased cyclic AMP response in sensitized guinea-pig lung in response to histamine stimulation. Hence, it is not known if the decreased cyclic AMP levels in rat mast cells is due to increased histamine release or if decreased cyclic AMP levels causes greater amounts of histamine to be released.

Rinard et al. (1979) showed that in dogs sensitive to ascaris, the isoproterenol-induced relaxation of trachealis muscle was less than in control animals. Furthermore, sensitized-dog trachealis had lower basal cyclic AMP levels than control trachealis and lower isoproterenol stimulated increases in cyclic AMP. These dogs had not been previously exposed to a β-agonist. Hence these authors ruled out desensitization of the β-receptor.

Sensitization increases the release of bronchoconstrictor PGs from the lung. Mathe et al. (1977) showed that ovalbumin sensitization

of guinea pigs caused significantly more $PGF_2\alpha$ in addition to histamine to be spontaneously released from the lung relative to control animals. Yen et al. (1978) obtained similar results. Rothberg & Hitchcock (1982) showed that microsomal fractions prepared from ovalbumin-sensitized guinea-pig lung synthesized more bronchoconstrictor TXA_2 and PGD_2 upon AA-induced stimulation than control microsomes. Boots et al. (1978) obtained similar results using perfused guinea-pig lungs. From these studies, it appears that immunological sensitization increases the production of bronchoconstrictor PGs in the airways.

Fish et al. (1981) showed that indomethacin-pretreatment of allergic asthmatic and allergic nonasthmatic (i.e. patients with allergic rhinitis) subjects, increased the sensitivity of the latter group to antigen-induced decreases in pulmonary function. Furthermore, sensitivity to methacholine was not changed by indomethacin. The authors concluded that PGs may be regulating mediator release in non-asthmatic subjects in a different fashion from asthmatics.

Walters et al. (1981) studied the effects of $PGF_2\alpha$-pretreatment on the pulmonary responses to histamine in normal subjects and showed that the sensitivity to histamine increased without a change in reactivity when the data was compared to placebo-pretreatment. However, opposite results were obtained by Fish et al. (1984) in asthmatic subjects. $PGF_2\alpha$-pretreatment decreased the response of human asthmatic airways to histamine. Also, experiments by Walters (1983) showed that immunization of normal humans against influenza

virus increased the sensitivity and decreased reactivity of the airways to histamine. Indomethacin-pretreatment abolished the increased sensitivity but increased reactivity, implying a role of PGs released in the inflammatory response to influenza virus in the induction of airway hyperresponsiveness.

1.3.3 Sensitization-dependent changes in the role of lipoxygenase metabolites:

Lipoxygenase products have been implicated in the pathogenesis of asthmatic hyperreactivity.

Increased synthesis of lipoxygenase products has been shown to occur as a consequence of immunological sensitization. This has been demonstrated in guinea-pig lung fragments (Piper & Seale, 1979) and in platelets from normal and asthmatic individuals (Yen & Morris, 1982). Furthermore, lipoxygenase products have been shown to increase release of histamine from human basophils (Peters et al. 1981).

The influence of prior exposure of airway tissue to lipoxygenase products on subsequent responses to bronchoconstrictors has also been studied. Generally, lipoxygenase products sensitize airway smooth muscle to the bronchoconstrictor effects of histamine and other contractile agonists. Copas et al. (1982) showed that 5-HETE contracted human isolated bronchial muscle and guinea-pig lung strips. 5-HETE at subthreshold doses also potentiated histamine-induced contractions of human airway muscle. LTD_4 and LTE_4 enhanced contractions of guinea-

pig tracheal spirals to histamine and acetylcholine (Creese & Bach, 1983; Lee et al. 1984). These effects are antagonized by FPL55712.

2. RESEARCH OBJECTIVES

2. RESEARCH OBJECTIVES

This thesis consists of three separate but related studies. Studies one and two utilize the actively-sensitized (ovalbumin as the antigen) guinea-pig model of asthma. The hypothesis explored in the first study was that immunological sensitization alters the responsiveness and sensitivity of airway tissues to bronchoconstrictors and bronchodilators. By analogy with human allergic bronchial asthma, immunological sensitivity to an antigen may result in bronchoconstrictor hyperreactivity and/or bronchodilator hypoactivity. These may arise as a consequence of altered AA metabolism (Yen & Morris, 1982), changes in receptor concentration (Szentivanyi, 1968; Kaliner et al. 1982), and/or changes in cellular regulatory processes of the airways (Mathe et al. 1978; Rinard et al. 1979).

In order to investigate such possible changes, the reactivity of sensitized airway tissue to bronchoconstrictors and bronchodilators was compared to appropriate unsensitized controls. Contractile responses and sensitivity of normal and sensitized isolated tracheal spirals (large airways) and lung parenchymal strips (small airways) to histamine, carbachol, LTC_4, LTD_4 and U-44069 were determined. These agents being chosen by virtue of their involvement in human asthma. Furthermore, the relaxant responses of normal and sensitized airway tissues to isoproterenol, PGE_2, forskolin and vasoactive intestinal polypeptide (VIP) were investigated. In conjunction with the relaxant effects of these bronchodilators, the responses of adenylate cyclase

in membranes prepared from normal and sensitized lung parenchyma were measured. This was done so as to obtain a direct index of receptor occupation and activation by these agents and to determine whether immunological sensitization alters tissue relaxation and/or cyclic AMP production. Again, this hypothesis was based on literature reports of changes in the effect of bronchodilator receptors and the adenylate cyclase complex in asthma. The compounds used in this study were chosen by virtue of the probable importance of the β-and VIP-receptor in asthma. Forskolin was used to obtain a non-receptor-mediated measurement of adenylate cyclase activation and PGE_2 because of the important modulatory function of this PG in the airways (Spannhake et al. 1981).

Since AA metabolites are synthetized and are active in airway tissues, the effects of inhibitors of the cyclooxygenase enzyme; i.e., indomethacin, the lipoxygenase enzyme; i.e., NDGA, and an inhibitor of both enzymes, i.e., phenidone, were investigated on the contractile responses of normal and sensitized tissues. The effects of indomethacin were also investigated on bronchodilator-induced relaxation of normal and sensitized airway tissues and on adenylate cyclase activation in normal and sensitized lung parenchymal membranes.

Various studies have suggested that AA metabolism is altered in allergic states. The use of inhibitors of AA metabolism in the above study served to define such a defect. This phenomenon was investigated more difectly by comparing A23187- induced and AA-induced

contractions of normal and sensitized airway tissues. Furthermore, the nature of lipoxygenase metabolite(s) released from the airways upon antigen, A23187, and AA challenge was investigated using reverse-phase high performance liquid chromatography techniques supplemented with UV spectrometry and bioassay techniques.

The nature of calcium regulation in the synthesis of the metabolite(s) mediating AA-induced contractions of the airways was also examined. This was done so as to determine whether calcium modulatory drugs preferentially antagonize mediator synthesis as opposed to smooth muscle contraction initiated by released mediators (See evidence presented in Section 1.2.3.3.4.). The third and final study in this thesis was to investigate the effects of LTD_4 on the guinea-pig lung and rat-cerebellar adenylate cyclase systems. The hypothesis that LTD_4 interacts with a receptor negatively linked to adenylate cyclase was tested (See evidence presented in Section 1.2.1.4.7). In this study adenylate cyclase was prepared from the above tissues and tested under conditions which were optimal for demonstration of inhibition of the enzyme.

3. MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Materials:

Histamine dihydrochloride, carbamylcholine chloride, nordihydroguaiaretic acid (NDGA), 1-phenyl-3-pyrazolidone (phenidone), adenosine, adenosine deaminase, vasoactive intestinal peptide (VIP), γ-amino butyric acid (GABA), arachidonic acid (AA), prostaglandin E_2 (PGE_2), and ovalbumin (OA, grade II for sensitization and grade V for challenge) were obtained from Sigma (St. Louis, MO). 8-(diethylamino)-octylo-3,4,5-trimethoxybenzoate hydrochloride (TMB-8)was from Aldrich Chemical Company (Milwaukee, WI). Forskolin, calcium ionophore A23187, GTP (trilithium salt), GTPγS, creatine kinase, dithiothreitol, creatine phosphate (potassium salt), AMP, cyclic AMP and ATP were from Calbiochem-Behring (San Diego, CA). [α-^{32}P]-ATP (25 Ci/mmole) was obtained from ICN chemicals (Irvine, CA).

LTC_4, LTD_4 and indomethacin were gifts from Drs. J. Rokach and Wm. D. Dorian, Merck Frosst Laboratories (Pointe Claire - Dorval, P.Q.). U-44069 was a gift from Dr. M. K. Bach, the Upjohn Company (Kalamazoo, MI).

All drugs were dissolved in water, except U-44069 (10^{-3} M) which was dissolved in 95% ethanol, indomethacin (10 mg ml^{-1}) in 1 M Tris buffer (pH 8.4) or ethanol, NDGA (100 mM) in water containing 1.5% NaOH to make a salt, A23187 (1 mg ml^{-1}) in ethanol, PGE_2 (10^{-2} M) in ethanol, forskolin (10^{-2} M) in DMSO, isoproterenol in water containing 100 μM ascorbic acid and AA (10 mg ml^{-1}) in 1 M Tris buffer (pH 8.4)

and stored in the dark at -70°C until used. LT stock solutions (0.2 mg ml^{-1}) were checked for purity by HPLC and the concentration adjusted to 10^{-4} M, and divided into aliquots and stored at -70°C until the day of use. Diluted solutions were stored at 4°C and used for a maximum of 1 week and then discarded.

The effect of vehicle was always tested on the responses being measured and it was ensured that none of the vehicles significantly affected the response.

All drugs were diluted in deionized water or in Krebs-Henseleit solution (KHS). Furthermore, drugs used in adenylate cyclase assays were always adjusted to pH 7.5 with Tris acid or base before storage or use.

All solvents used in HPLC were of HPLC grade. All other drugs, chemicals and solvents were at least of reagent quality.

3.2 METHODS

3.2.1 Sensitization of animals and preparation of tissues:

Male English short-hair guinea pigs (200-250 g) (Connaught Laboratories, Toronto, Ontario) were actively sensitized with ovalbumin (OA) (grade II) 100 mg s.c. and 100 mg i.p. Normal animals were untreated and age and weight matched with sensitized animals in all experiments. Animals were killed by stunning and exsanguination (3-4 weeks after OA injection for sensitized animals). The trachea and lung were removed immediately and placed in KHS. The composition

(mM) of the KHS was NaCl, 118; KCl, 4.7; $MgSO_4.7H_2O$, 1.2; $CaCl_2$, 2.2; KH_2PO_4, 1.2; $NaHCO_3$, 24.9; D-glucose, 11.1. The trachea was cut spirally (Constantine, 1965) and divided into four equal segments so that one always acted as a control to detect changes occurring as a result of time, vehicle, or previous additions of drugs. Parenchymal strips were prepared from the distal edges of each lung lobe (Lulich et al. 1976). Each strip was approximately 3 x 3 x 30 mm. Four tissues were obtained from each animal and used as above. Tissues were placed in water-jacketed 10 ml baths, maintained at 37°C in KHS aerated with 95% O_2 and 5% CO_2. Tissues were attached by silk threads to force displacement transducers (Grass FT03C) and the responses displayed on Grass polygraphs (Model 7D). An initial load of 1 g was placed on each tissue. At the end of a 90 min equilibration period, during which the bath fluid was replaced with fresh KHS every 15 min, the resting tone was adjusted to 1 g. Initial experiments with KCl-induced contractions (Fig. 3) demonstrated that the length-tension relationships were similar for both normal (N) and sensitized (S) tissues. The optimal tension was 1.0 - 1.5 g for both trachea and parenchyma. Higher applied tension, e.g., 2 g tended to tear parenchymal strips. Tracheal spirals normally increased in tone whereas parenchymal strips tended to relax slightly under 1 g applied tension. The dry weight of each tissue was determined at the end of the experiment (after drying overnight at 60°C). The dry weight was used to normalize the force developed to the smooth muscle mass (Brink

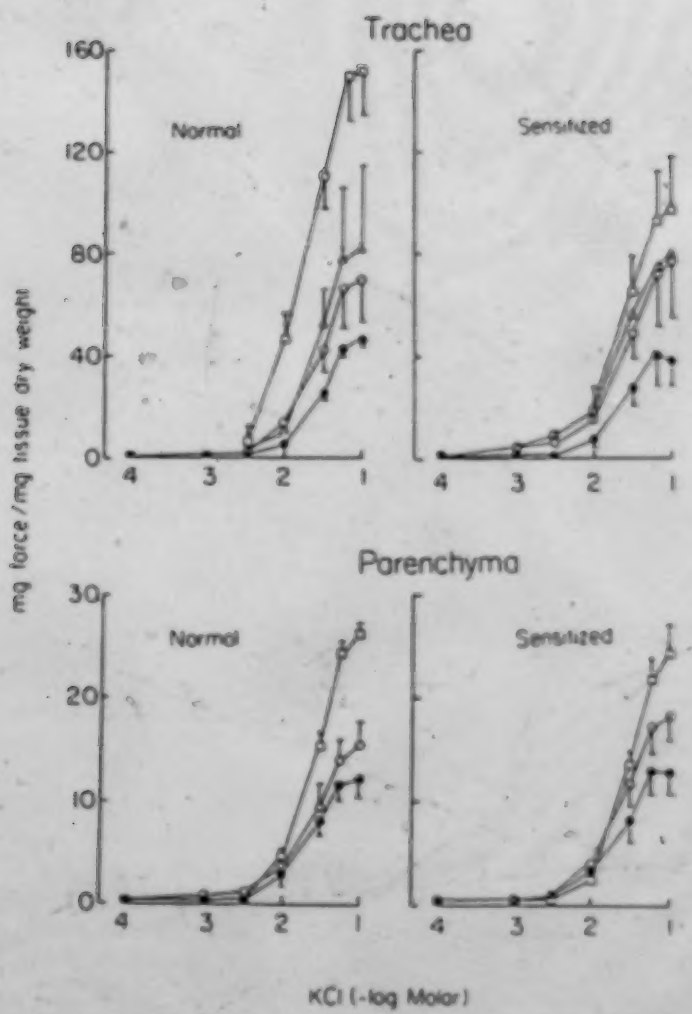


Fig. 3. Length-tension relationships of isolated guinea pig airways. Contractile responses of normal and sensitized tissues to KCl are shown. Resting tension imposed upon the tissues was 0.5 g (●), 1.0 g (○), 1.5 g (□), and 2.0 g (△). The results are the mean±SEM (n=3).

et al. 1980). This normalization assumes that the percentage of smooth muscle in trachea and parenchyma is constant. Furthermore, the maximal response to histamine (100 μM) was determined initially and other responses expressed as a percentage of this value.

3.2.2 Concentration-response curves to bronchoconstrictors and bronchodilators: Effects of cyclooxygenase and lipoxygenase inhibitors on responses to agonists:

After the responses to histamine (100 μM) became constant, concentration-response (C-R) curves were established for histamine, carbachol, LTC_4, LTD_4 and U-44069 on N and S tracheal spirals and parenchymal strips. Only one bronchoconstrictor was studied on tissues from any one animal. Additions of drugs were made in graded dose form given in random order with two exceptions; (i) on tracheal spirals U-44069, LTC_4, and LTD_4 were added in cumulative fashion since the tissue responses to these agonists were very prolonged and difficult to reverse by washing (in initial experiments we did not note any differences in C-R curves achieved by graded dose or cumulative addition); (ii) on parenchymal strips the LTs were added in graded doses given in increasing concentrations. These agonists were not added randomly since the tissues desensitized completely after high concentrations. Only one C-R curve was generated using these agonists on the parenchyma.

After establishment of the initial C-R curves, the tissues were

incubated for 30 min with indomethacin (8.5 μM), NDGA (30 μM), or phenidone (185 μM). These concentrations effectively inhibit the cyclooxygenase (indomethacin, phenidone) and lipoxygenase (NDGA, phenidone) enzymes (Vane, 1971; Blackwell and Flower, 1978; Morris et al. 1979). The fourth control tissue was incubated with vehicle in the final concentration used with the test tissues. The inhibitors were kept in the bath upon reestablishment of the second C-R curves to the bronchoconstrictors. Hence, all tissues had two curves generated on them except for LTC_4 and LTD_4 on lung parenchyma where only one curve was generated on control and inhibitor treated tissues, immediately after establishment of the optimal response to histamine (100 μM).

When sensitized tissues were used, they were challenged with OA (1 μg/ml) at the end of the experiment to confirm that the sensitization procedure was effective.

The increases in force as a result of drug addition were determined from the recorded responses and normalized in two ways: (i) after dividing by the tissues dry weight and (ii) by expressing as a percentage of the maximum response to histamine determined initially for every tissue before the addition of inhibitors. The effects of inhibitors on tissue tone was expressed as percentage change from preincubation tone.

The concentration of an agonist required to increase tension by 50% of the maximum attained and by 50% of the maximum attained by

histamine were the EC_{50} and EC_{50} maximum histamine, respectively. These values were extrapolated from hand-fitted individual C-R curves. The negative logarithm of the EC_{50} is the pD_2 and is an indication of the potency of an agonist. The EC_{50} maximum histamine values were also converted into negative logarithms. Data was analyzed for statistical significance by the use of Student's t-test for paired and unpaired data as appropriate. The difference between groups was considered significant when $p < 0.05$.

The relaxant effects of isoproterenol, forskolin, PGE_2, and VIP were determined on N and S tissues prepared as above. Tracheal spirals were pre-contracted with carbachol (1 μM) a concentration which produces 70-80% of the maximum response. Parenchymal strips were not precontracted but mechanically adjusted to maintain 1 g baseline tension. The agents were added cumulatively until the maximum response reached a plateau at which isoproterenol (10 μM) was added to fully relax the tissue. Responses were expressed as percentage maximum response to isoproterenol (10 μM), and as mg relaxation/mg dry weight of tissue. Both forms of data expression gave similar conclusions. Hence, only the former are presented.

Two relaxant drugs were studied on tissues from one animal. One acted as a paired time control and the other to determine the effect of indomethacin (8.5 μM) on the reestablished C-R curves to the above relaxants.

All comparisons (to detect the effects of inhibitor treatment)

were made with the appropriate time and vehicle control tissue. Further analyses were as described for bronchoconstrictors.

3.2.3 Responses to antigen, ionophore A23187 and AA. Effects of AA metabolism inhibitors and calcium-modulatory treatments:

Tissues from N and S animals were prepared as described in section 3.2.1. Tissues were treated with indomethacin (8.5 μM), NDGA (100 μM) or vehicle. After treatment of tissues with the above inhibitors for 30 min, the tissues were challenged with A23187 (5.7 μM) or ovalbumin (3 μg/ml). A23187 was added to both normal and sensitized tissues. AA (66 μM) was added to some tissues 1 min before the above stimuli.

In further experiments the effects of mepacrine (210 μM), a phospholipase inhibitor, were also investigated. The protocol in these experiments is shown in Table 4.

In the above experiments, the bath fluid was collected at the end of 60 min and LTs extracted as described later in section 3.2.4.

In further experiments to investigate the effects of Ca^{2+} modulatory treatments on AA-induced contractions, the following experiments were performed on normal airway tissues only. Tissues were set up as described earlier and pretreated with indomethacin (8.5 μM) for 30 min. They were then incubated as shown in table 5 for 30 min prior to the addition of AA (66 μM). In experiments 6, 7, 8 and 9, AA-induced contractions were measured for 60 min after which

Table 4. Experimental protocol for the investigation of the effects of mepacrine and NDGA on A23187- and ovalbumin-induced contractions of indomethacin (8.5 μM)- pretreated N and S tracheal spirals and lung parenchymal strips.

Experiment	Modulatory agent (30 min incubation)	Stimulus
1	None	A23187 (5.7 μM) or OA (3 $\mu g ml^{-1}$)
2	None	AA (66 μM)
3	Mepacrine (210 μM)	AA (66 μM)
4	Mepacrine (210 μM)	A23187 (5.7 μM) or OA (3 $\mu g ml^{-1}$) and AA (66 μM)
5	Mepacrine (210 μM)	A23187 (5.7 μM) or OA (3 $\mu g ml^{-1}$)
6	NDGA (100 μM)	AA (66 μM)
7	NDGA (100 μM)	A23187 (5.7 μM) or OA (3 $\mu g ml^{-1}$) and AA (66 μM)

Table 5. Experimental protocol for Ca^{2+} studies. Tissues in Ca^{2+}-free KHS were washed frequently during the prechallenge period to ensure removal of extracellular Ca^{2+}.

All tissues challenged with AA (66 μM)

Experiment	Ca^{2+} concentration in bath	Treatment (30 min)
1	2.2 mM (control)	None
2	2.2 mM	Lanthanum chloride (1 mM)
3	2.2 mM	Nitrendipine (1,10 or 100 μM)
4	2.2 mM	Verapamil (100 μM)
5	8.8 mM	None
6	Ca^{2+}-free	None
7	Ca^{2+}-free	Lanthanum chloride (1 mM)
8	Ca^{2+}-free	EDTA (300 μM)
9	Ca^{2+}-free	TMB-8 (100 μM)

Ca^{2+} (2.2 mM) was added and the contractions measured for a further 60 min.

Cumulative C-R curves to LTC_4 were also constructed on indomethacin (8.5 μM)-pretreated tissues in normal KHS (control), in Ca^{2+}-free KHS with and without lanthanum chloride (1 mM) and in normal KHS in the presence of nitrendipine (100 μM). Ca^{2+} (2.2 mM) was added to all tissues after the maximum response to LTC_4 (0.1 μM) was obtained. All response were expressed as a percentage of the maximum response to histamine (100 μM) obtained in normal KHS.

3.2.4 Extraction, initial purification, reverse-phase high performance liquid chromatography, ultraviolet spectrometry and bioassay of leukotrienes released from airway tissues:

After 1 hour of the release reaction induced by OA, A23187 or AA (section 3.2.3), the bath fluid was collected and kept on ice. The pH was adjusted to 3 with 1N HCL and the solution passed through a 0.22 μm filter (MILLEX-GS, Millipore). The filtered solution was then passed through a primed SEP-PAK C_{18} cartridge (Waters Associates) Priming was carried out by washing the cartridge with 5 ml methanol followed by 40 ml water. The loaded cartridge was successively washed with 5 ml water, 5 ml water/methanol (65:35 vol:vol) and 5 ml methanol. The last fraction was collected and the methanol blown off at 50°C under a nitrogen stream. Experiments with added [^{3}H]-LTC_4 standard indicated that this fraction contained about 80% of the

initial LTC_4 in the sample (Table 6). Furthermore, none of the drugs used interfered with the recovery of LTs.

The residue left after evaporation of methanol was suspended in a small volume (200 μl) of distilled water and used in high performance liquid chromatography (HPLC) or bioassay procedures.

The final purification and analysis of samples was carried out by HPLC using a C_{18} column (Radial-pak, Waters Associates). Elution was carried out using methanol/water/acetic acid (65:35:0.1, vol/vol) (pH 5.5, adjusted with ammonium hydroxide). The flow rate was 1 ml min^{-1} and column back pressure was 1000 psi. Eluants were continuously monitored at 280 nm. Synthetic LTC_4 and LTD_4 were injected as standard. Peaks in the samples corresponding to the retention times of LTC_4 and LTD_4 were collected (Fig. 4), the pH adjusted to neutrality with 1 N NaOH, evaported to dryness, resuspended in water and rechromatographed.

Rechromatrographed samples were concentrated as above and suspended in methanol for ultraviolet spectrometry on a Carey 15 spectrophotometer. Bioassay was carried out on the atropinized (1 μM) and mepyraminized (1 μM) guinea-pig ileum longtitudinal strip and lung parenchymal strip. Positive contractions were reversed with FPL55712 (0.17-8.7 μM). Synthetic LTC_4 was used as a standard in the above bioassay procedures. Further experiments with an LTC_4-specific radioimmunoassay carried out by Dr. Burka confirmed the identity of the above mediators.

Table 6. Percentage recovery of [^{3}H]-LTC_4 utilizing C_{18}-SEP-PAKs[a]

Condition	% of initial radioactivity
KHS	2.8 ± 0.8[b]
Water wash	0.0 ± 0.0
35% Methanol wash	7.7 ± 1.1
100% Methanol wash	79.0 ± 2.6
Cartridge	8.2 ± 2.5

[a]number of experiments = 12

[b]values are mean ± SEM

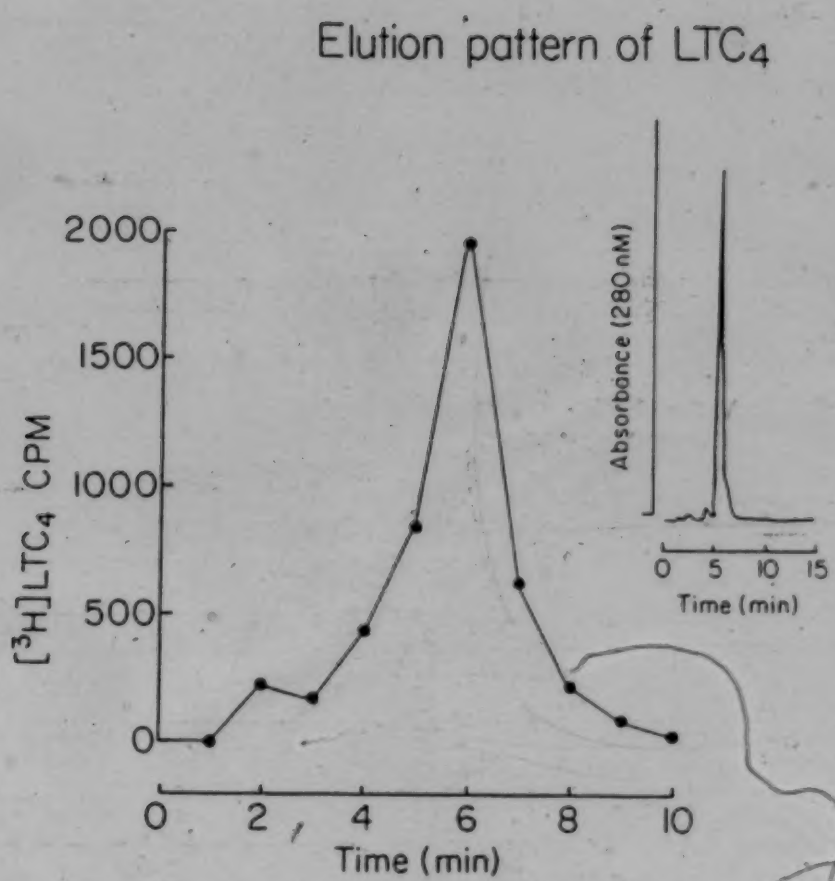


Fig. 4. High performance liquid chromatorgraphy of LTC_4 monitored by liquid scintillation counting of [^{3}H]-LTC_4 (main figure) and by UV absorbance at 280 nm (Inset). Elution times were similar.

3.2.5 Adenylate cyclase assay procedures:

Two studies involving measurement of adenylate cyclase activity were performed in this thesis. The first study attempted to determine whether adenylate cyclase activation induced by various bronchodilator drugs differed between N and S guinea-pig lung parenchymal tissue. Due to the inherent difficulty in obtaining hormone-sensitive adenylate cyclase from pure smooth muscle preparations (Muller, 1985), guinea-pig trachea was not studied in this thesis.

The second study of adenylate cyclase carried out in this thesis involved investigating the effects of LTD_4 on guinea-pig lung and rat cerebellar cyclases. The methods used in studies 1 and 2 differed slightly. These differences will be pointed out as appropriate.

3.2.5.1 Preparation of membranes:

Lungs from two animals were used for each membrane preparation. Whole lungs were removed from animals and perfused through the pulmonary artery with ice cold KHS until the perfusate was free of blood. The lungs were then chopped (excluding large bronchi, fat and adhering tissue) on ice. The chopped lung was then divided equally into two portions, placed in oxygenated KHS (30 ml) and incubated at 37°C for 30 minutes. One portion was treated with indomethacin (8.5 μM) and the other with vehicle for 30 min. Hence, experiments were done on a paired basis. The above incubation procedure was not followed in the second adenylate cyclase study. The lung portions

were then homogenized at 4°C in a glass homogenizer with a motor driven Teflon pestle (300 rpm) for 1 min in 10 volumes of homogenization buffer of the following composition: Tris-HCl (50 mM), dithiothreitol (1 mM) and sucrose (0.25 M) pH 7.5 at 4°C. The homogenate was filtered through glass wool and cheese cloth and centrifuged at 500 x g for 10 minutes at 4°C. The pellet made up of tissue fragments, unbroken cells and nuclei was discarded. The supernatant was subsequently centrifuged at 25,000 x g for 20 min at 4°C. The resultant pellet was resuspended in buffer identical to the homogenization buffer except not containing sucrose, and centrifuged again at 25,000 x g for 20 min at 4°C. The resultant pellet was gently resuspended in the above buffer with a hand-held teflon pestle in a glass homogenizer. Protein concentration was adjusted between 0.5-3.0 mg/ml. Protein was determined by the method of Lowry et al. (1951). Aliquots of 500 µl were frozen and stored (less than four weeks) until use under liquid nitrogen.

Essentially the same procedure was followed for preparation of rat cerebellar adenylate cyclase. Male Sprague-Dawley rats (300-500 g) were used in these experiments.

3.2.5.2 Adenylate cyclase assay procedure:

The procedure followed was that of Baer (1975). This method uses polyethyleneimine thin layer chromatography plates (Macherey-Nagel/ Brinkman Instruments, Rexdale, Ontario), and development in lithium

chloride (0.25 M) to separate cyclic AMP from ATP. Assays were conducted in duplicate or triplicate in a final volume of 50 μl. Each tube contained the following: 25 mM 4-(2-hydroxyethyl)-1-piperazine-ethane sulphonic acid (HEPES, pH 7.5) or Tris acid - Tris base (25 mM, pH 7.5), 5 mM $MgCl_2$ (for study 1, $MgCl_2$ or $MnCl_2$ at various concentrations in study 2 as indicated in results), 1 mM cyclic AMP, 10 mM creatine phosphate, 0.2 mg ml^{-1} creatine kinase, 0.1 mM [α-^{32}P]-ATP (500,000-800,000 cpm) and 5-50 μg membrane protein (the reaction was always shown to be linear under various conditions. See results). Drugs and other additions or changes in the conditions are shown under the appropriate results.

Incubations were carried out at 20°C and 37°C for various times (3-40 min) as indicated in results. The reaction was stopped by adding 10 μl of a "stop" solution (pH 7.5) containing sodium salts of cyclic AMP, ATP, AMP, and EDTA, each at 25 mM. Alternatively for time course studies, 5 μl of a 3 fold water diluted "stop" solution were spotted on water-washed thin layer plates (20 x 10 or 20 x 6.5 cm) and a 5 μl aliquot of the adenylate cyclase reaction mixture spotted on top of this at the appropriate time.

The plates were then developed in LiCl (0.25 M). Spots were visualized under ultraviolet light and those corresponding to "ATP plus 5'-AMP" and "cyclic AMP" were cut out in a fume hood and placed in scintillation vials containing 10 ml scintillation fluid (Toluene containing PPO (4 g/l) and POPOP (0.2 g/l). The ratio of cpm in the

cyclic AMP and ATP + 5'AMP spots provided a measure of percent conversion of ATP to cyclic AMP. These values were calculated by a computer program after substraction of blank values (assays carried out in the absence of enzyme determined with every experiment).

The results are reported as the mean ± SEM of duplicate or triplicate determinations which never varied by more than 5-10%. Enzyme activity was calculated as cyclic AMP formed (pmoles/min/mg protein) or as percentage conversion values.

4. RESULTS

4.1 RESPONSES OF NORMAL AND SENSITIZED AIRWAY TISSUES TO BRONCHOCONSTRICTORS AND BRONCHODILATORS

4.1.1 Contractile responses to bronchoconstrictors:

The forces generated by tracheal spirals and parenchymal strips after additions of histamine, carbachol, U-44069, LTC_4, and LTD_4 were similar in normal and sensitized guinea pigs (Fig. 5). Since increases in force over baseline tension after additions of histamine were not different in normal and sensitized tissues, all the data were normalized by expressing responses as a percent of the histamine maximum on each tissue obtained in the absence of any modulatory drug (100 μM histamine=100%). The resulting C-R curves were less variable but equivalent to those expressed in absolute force units; i.e., mg force per mg tissue dry weight, data not shown. Histamine was chosen as the reference agonist because of the potential importance of this mediator in allergic asthma.

On the trachea the cholinergic agonist carbachol induced the greatest increase in force at the maximum concentration used (30 μM). Histamine was the next most effective agent followed by the endoperoxide analog U-44069 (Table 7). The C-R curves to the latter agent had a biphasic profile with an initial plateau at about 1 nM. This effect was observed in both normal and sensitized tracheal spirals. LTC_4 and LTD_4 induced the least increases in force when added at concentrations of 0.1 μM, the maximum we could use due to limited availability of these synthetic agents. LTC_4 was equiactive

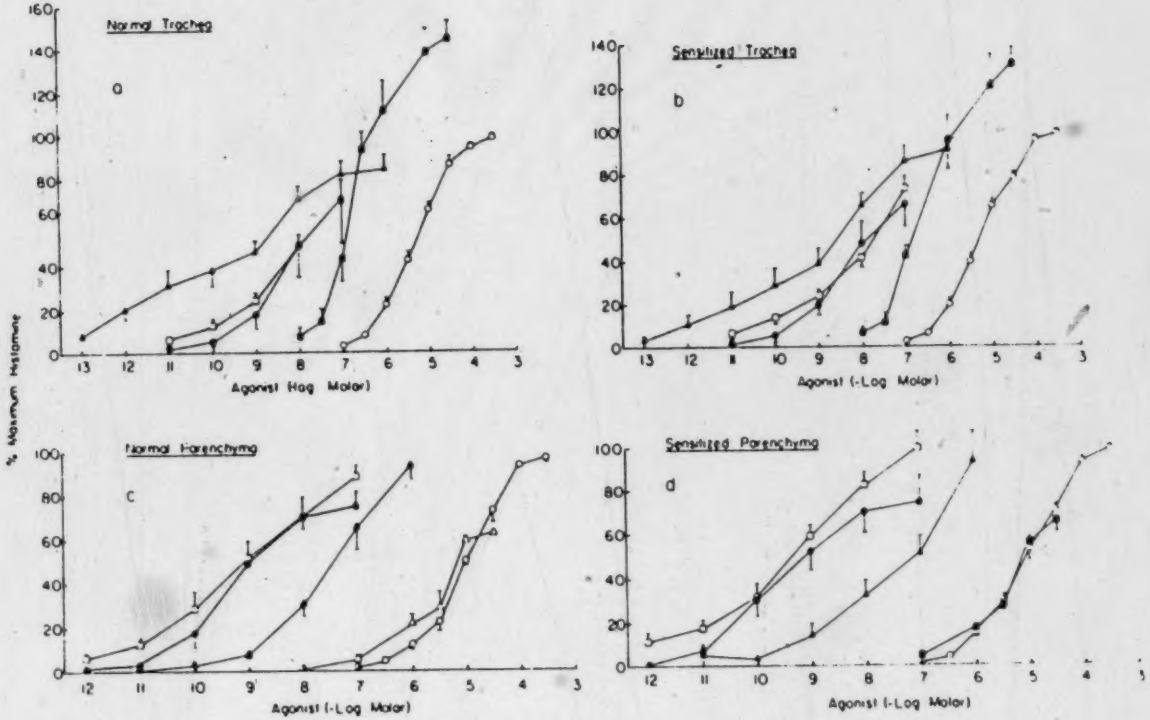


Fig. 5. Concentration-response curves to bronchoconstrictors in tracheal spirals (a and b) and parenchymal strips (c and d) from normal and ovalbumin-sensitized guinea pigs. Values are the mean ± SEM (n=number of animals used for each drug and is shown for normal trachea, sensitized trachea, normal parenchyma, and sensitized parenchyma, respectively after each drug symbol). Bronchoconstrictors were histamine (○) (13,13,10,13), carbachol (■) (6,6,6,10), leukotriene C_4 (●) (3,3,3,3), leukotriene D_4 (□) (5,11,10, 9), and U-44069 (▲) (6,6,5,6).

Table 7. Increases in tension over baseline after the addition of bronchoconstrictor agonists to normal and sensitized airway tissue.

	Trachea		Parenchyma	
Agonist	Normal	Sensitized	Normal	Sensitized
Histamine (300 μM)	154.5±22.9 (13)	166.2±19.6 (13)	13.2±1.9 (10)	11.4±0.94 (13)
Carbachol (30 μM)	288.5±43.0 (6)	256.0±43.0 (6)	8.0±1.7 (5)	6.7±0.6 (10)
U-44069 (1 μM)	152.7±14.6 (6)	159.8±37.2 (6)	9.7±1.2 (5)	11.6±3.1 (6)
LTC_4 (0.1 μM)	129.7±33.9 (3)	102.3±16.3 (3)	9.1±3.6 (3)	7.3±1.3 (3)
LTD_4 (0.1 μM)	126.1±7.42 (5)	140.6±28.0 (6)	9.32±0.88 (10)	9.6±1.2 (9)

NOTE: Mean ± SEM, values in parentheses are number of animals. Values are in mg force per mg of tissue dry weights.

to LTD_4 on normal and sensitized trachea.

Histamine induced a greater contractile force on lung parenchymal strips than carbachol. Since U-44069, LTC_4 and LTD_4 were not used at concentrations sufficiently high to cause maximum responses, their efficacies could not be compared. Nonetheless, at the maximum concentrations used (Figs. 5c, 5d) these agents increased force to levels close to the histamine maximum response (Table 7).

The threshold concentrations of the above agents, as extrapolated from C-R curves, were similar in the appropriate tissues of normal and sensitized animals. However, differences were noted between the trachea (large airways) and the lung parenchyma (small airways). U-44069 was bronchoconstrictor on the trachea at concentrations as low as 10^{-13} M, whereas, on the parenchyma, a contractile effect was observed at about 10^{-11} M. LTD_4 induced contractile effects on the parenchyma at 10^{-12} M, but only caused an equivalent effect (percent histamine maximum) on the trachea at a concentration of 10^{-11} M (Fig. 5).

4.1.2 Sensitivity of tracheal and parenchymal strips to bronchoconstrictors:

The pD_2 and -log EC_{50} histamine maximum values for histamine, carbachol, U-44069, LTC_4, and LTD_4 on normal and sensitized airway tissues are shown in Table 8. There was no observable difference in sensitivity of the airways to the above bronchoconstrictors between

Table 8. Sensitivity of airway tissues from normal and sensitized animals to bronchoconstrictor agents *in vitro*.

	Trachea		Parenchyma	
Agonist	Normal	Sensitized	Normal	Sensitized
Histamine	5.34±0.08[a] (13)	5.4 ±0.08[a] (15)	5.01±0.04[a] (10)	5.17±0.09[a] (14)
Carbachol	6.41±0.13[a] (6)	6.59±0.09[a] (6)	5.58±0.05[a] (10)	5.45±0.11[a] (6)
U-44069	9.7±0.3[a] (5)	9.1 ±0.3[a] (6)	7.14±0.17[b] (6)	7.21±0.24[b] (6)
LTC_4	8.2±0.3[b] (3)	8.17±0.2[b] (3)	8.89±0.06[b] (3)	8.96±0.6[b] (3)
LTD_4	7.82±9.26[b] (5)	7.86±0.15[b] (10)	8.9 ±0.25[b] (10)	8.85±0.28[b] (10)

NOTE: Mean ± SEM. values in parentheses are number of animals.

[a] pD_2 = -log EC_{50} maximum of drug.

[b] -log EC_{50} histamine maximum.

normal and ovalbumin-sensitized animals as determined in vitro (Fig. 5, Table 8). The order of potency of the agonists on normal and sensitized trachea was U-44069 > LTC_4 > LTD_4 > carbachol > histamine. On the parenchyma the order was LTC_4 = LTD_4 > U-44069 > carbachol > histamine.

In all our experiments, challenge of sensitized tissues with OA resulted in a prolonged dose-dependent contraction. Concentrations as low as 0.1 μg/ml were effective with a maximum obtained at about 10 μg/ml. Addition of OA (100 μg/ml) to normal tissues did not induce any observable effects. Hence the basic criterion of hypersensitivity to a specific sensitizing antigen was fulfilled.

4.1.3 Effects of cyclooxygenase and lipoxygenase inhibitors on the responsiveness and sensitivity of airways to bronchoconstrictors:

Indomethacin (8.5 μM), NDGA (30 μM), and phenidone (185 μM) reduced the tone of tracheal spirals but had no effect on the tone of parenchymal strips (Table 9). The tone of tracheal spirals was reduced most markedly by phenidone and least by NDGA. Tone of normal and sensitized tracheal spirals were reduced to a similar extent. The effects of indomethacin, NDGA, and phenidone on responses of the trachea to histamine and carbachol are shown in Fig. 6. For both of these bronchoconstrictors the tension developed to low concentrations was reduced whereas tension developed to higher and maximal

Table 9. Effects of inhibitors on tone and percentage change from preincubation tone.

	Trachea		Parenchyma	
Inhibitors	Normal	Sensitized	Normal	Sensitized
Indomethacin (8.5 μM)	-25.9±3.4% (21)	-29.9±5.9% (26)	-4.1±0.7% (14)	-4.2±0.5% (15)
Phenidone (185 μM)	-44.5±5.4% (21)	-41.9±3.7% (25)	-4.8±1.0% (14)	-5.5±0.7% (16)
NDGA (30 μM)	-15.3±4.3% (22)	-12.2±5.6% (25)	-3.5±0.7% (14)	-3.5±0.9% (15)
Vehicle (control)	±23.8±5.6% (22)	+18.7±6.4 (26)	-2.8±0.5% (14)	-2.6±0.97% (15)

NOTE: Mean ± SEM, values in parentheses are number of animals.

*$p < 0.01$ versus control.

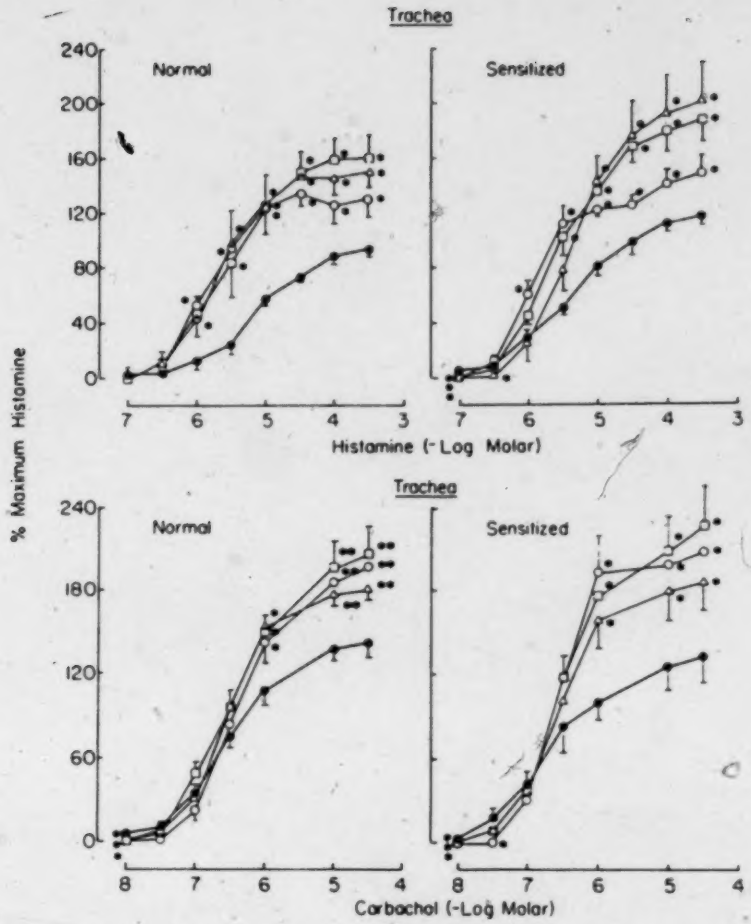


Fig. 6. Concentration-response curves to histamine (upper panel) and carbachol (lower panel) on normal and sensitized guinea-pig tracheal spirals, after pretreatment with indomethacin (8.5 μM) (□), NDGA (30 μM) (△), phenidone (185 μM) (○), or the paired control tissue (●). Values are mean ± SEM (n=5 for each curve). Significant differences from paired control responses are as indicated: *$p < 0.05$ and **$p < 0.01$.

concentrations was enhanced significantly ($p<0.05$) when compared with the appropriate paired control tissue. The responses in the presence of the inhibitors were not different between normal and sensitized tissues.

Figure 7 shows the responses of the trachea to LTC_4 and LTD_4 after incubation with the inhibitors. There was a reduction in responses to low concentration of these agents in both normal and sensitized tissue. There was also a tendency towards enhancement to higher concentrations of these agents although this was not statistically significant due to large variability of the observations.

The responses of the trachea from normal and sensitized animals to the endoperoxide analog U-44069 after incubation with indomethacin (0.28, 2.8, and 8.5 μM) are shown in Fig. 8. The responses to lower concentrations of U-44069 were unaffected by prior and continued exposure to indomethacin. On normal trachea the responses to higher concentrations of U-44069 were significantly enhanced ($p<0.05$) after exposure to indomethacin (2.8 and 8.5 μM), whereas no such enhancement was observed on sensitized tracheal spirals. Furthermore, the enhanced responses of normal trachea were significantly ($p<0.05$) different from the comparable responses on sensitized tissues.

The responses of normal and sensitized lung parenchymal strips to histamine, carbachol, and U-44069 were not affected by prior treatment with the inhibitors (Fig. 9).

LTC_4 and LTD_4 C-R curves were not reproducible on the parenchyma

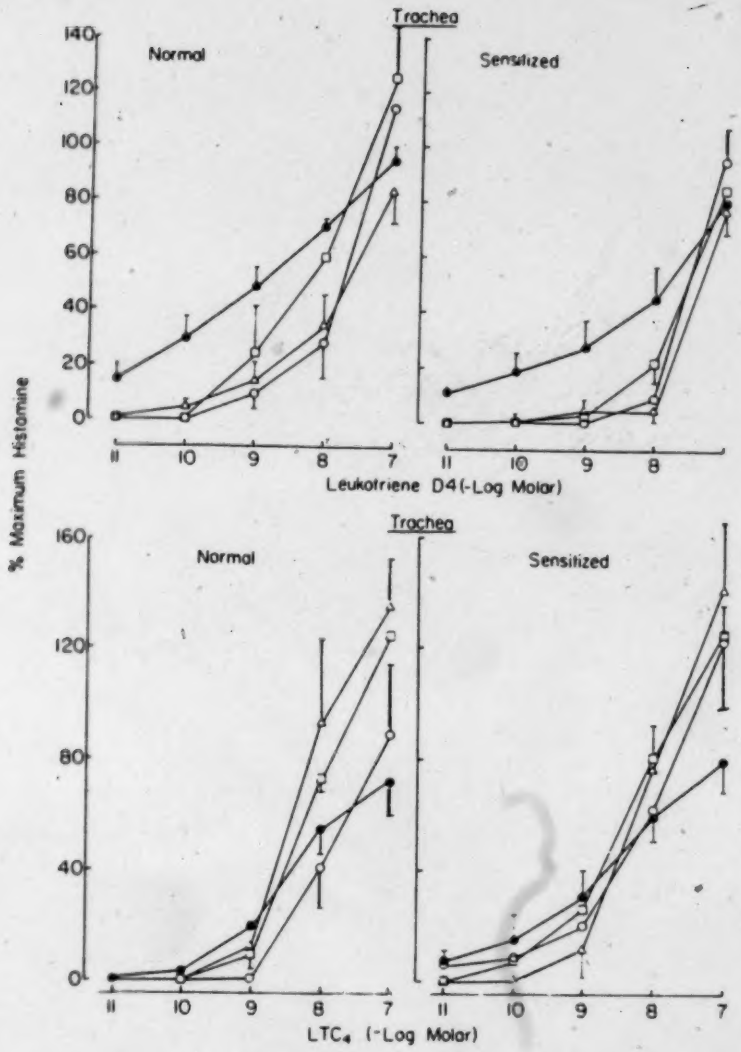


Fig. 7. Concentration-response curves to leukotrienes C_4 and D_4 on normal and sensitized guinea pig tracheal spirals after pretreatment with indomethacin (8.5 μM) (□), NDGA (30 μM) (△), phenidone (185 μM) (○), or the paired control tissue (●). Values are mean ± SEM (n=3 animals/curve for leukotriene C_4 and 6 animals/curve for leukotriene D_4).

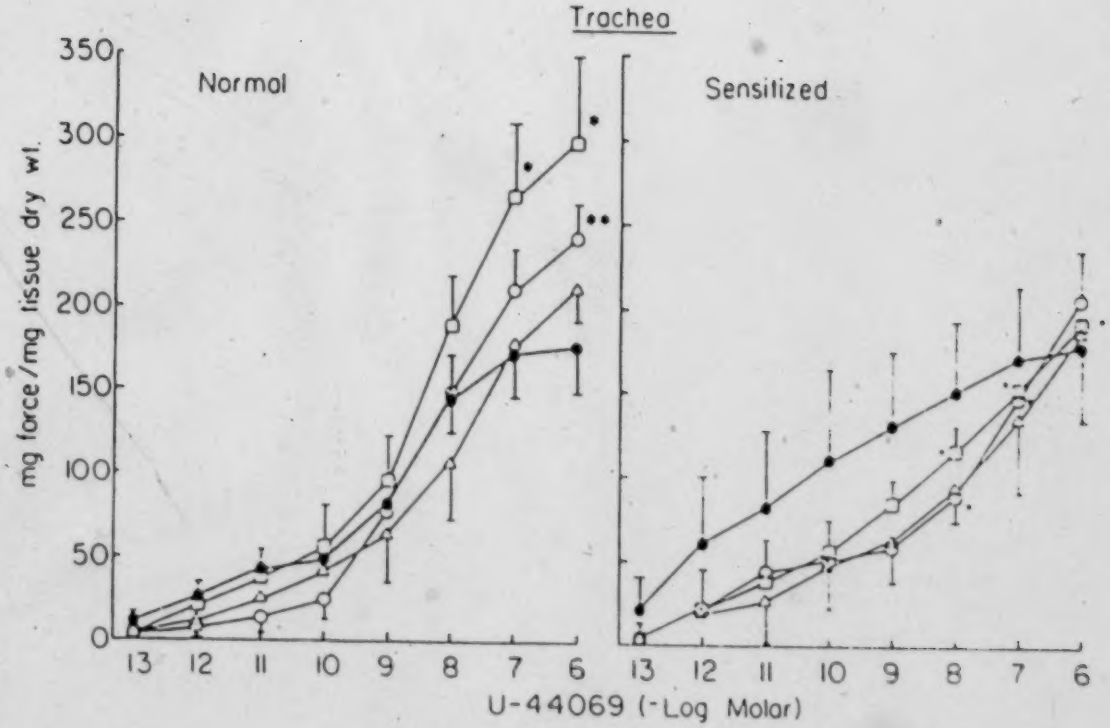


Fig. 8. Concentration-response curves to U-44069 on normal and sensitized tracheal spirals after pretreatment with indomethacin (0.28 μM) (△), (2.8 μM) (○), (8.5 μM) (□), or the paired control tissues (●). Responses are mean±SEM of the absolute force developed over resting tone, divided by the tissue dry weight (n=5 animals for each curve). Significant differences from paired control responses are as indicated: *p<0.05, **p<0.01. Significant differences between similarly treated tissues from normal and sensitized guinea pigs are also indicated in the sensitized tissues panel (*p<0.05).

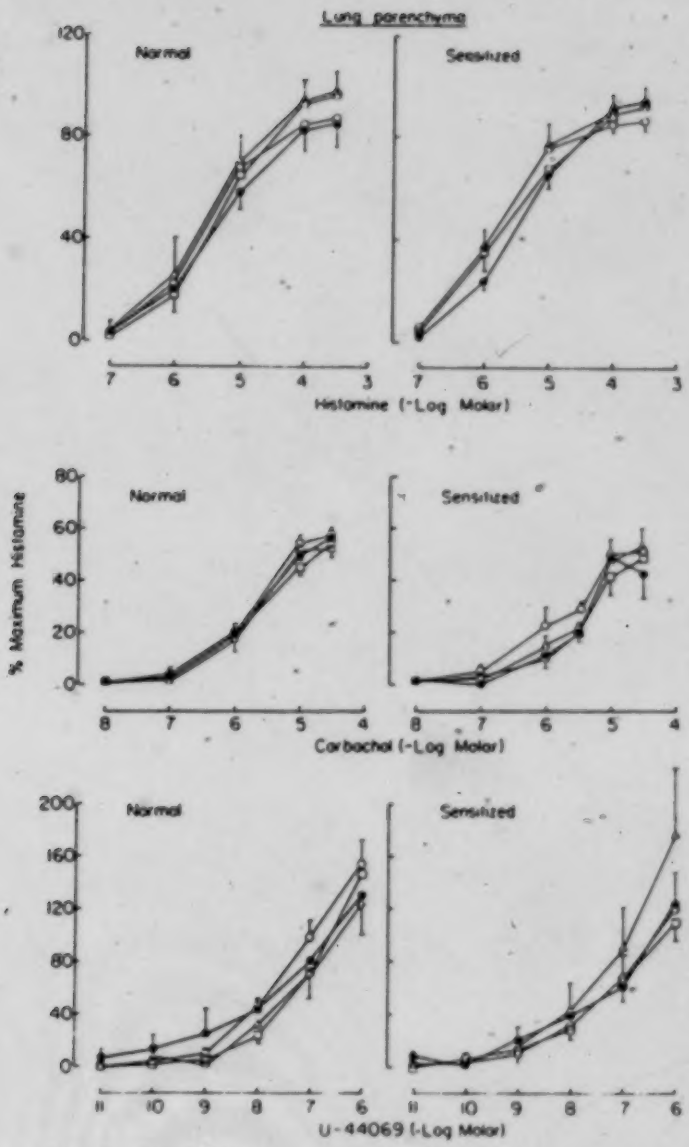


Fig. 9. Concentration-response curves to histamine (top panel) and carbachol (middle panel) on normal and sensitized guinea pig lung parenchymal strips after pretreatment with indomethacin (8.5 μM) (□), NDGA (30 μM) (△), phenidone (185 μM) (○), or the paired control tissue (●). Responses to U-44069 (bottom panel) after indomethacin pretreatment (0.28 μM) (△), (2.8 μM) (○), (8.5 μM) (□), or the paired control responses (●) also are shown. Results are presented as mean±SEM (n>5 for each curve).

as mentioned earlier. The tissues were primed, then incubated with the inhibitors (vehicle for the paired control tissue), and then one C-R curve was generated on each tissue. Figure 10 shows the response of normal and sensitized parenchyma to LTC_4 and LTD_4 after the above treatment. All the inhibitors reduced the contractile response to high concentrations of LTC_4 and LTD_4 (10^{-10} to 10^{-7} M). Responses to low concentrations of these agonists (10^{-12}, 10^{-11} M) were also reduced.

Concentration-response curves for the bronchoconstrictors in the presence of modulatory drugs were the same if established from the reduced basal tone which is a consequence of these drugs or if established from a tone readjusted mechanically to 1 g tension. This ensured that changes in the responsiveness and sensitivity, as a consequence of cyclooxygenase and lipoxygenase inhibitors, were not due to changes in tone.

The sensitivities of normal and sensitized trachea to histamine, carbachol, and U-44069 were not signfiicantly different from those of subsequently generated paired time-control curves. The pD_2 values from the initial C-R curves for histamine and carbachol on normal trachea were 5.47±0.13 (n=8) and 6.57±0.08 (n=10) (mean±SEM), whereas the comparable pD_2 values derived after generation of a second C-R curve were 5.21±0.06 (n=8) and 6.52±0.09 (n=10) ($p>0.05$).

The pD_2 values for histamine were unchanged after treatment with indomethacin, phenidone, and NDGA on normal and sensitized trachea (Table 10). Sensitivity of normal and sensitized trachea to carbachol

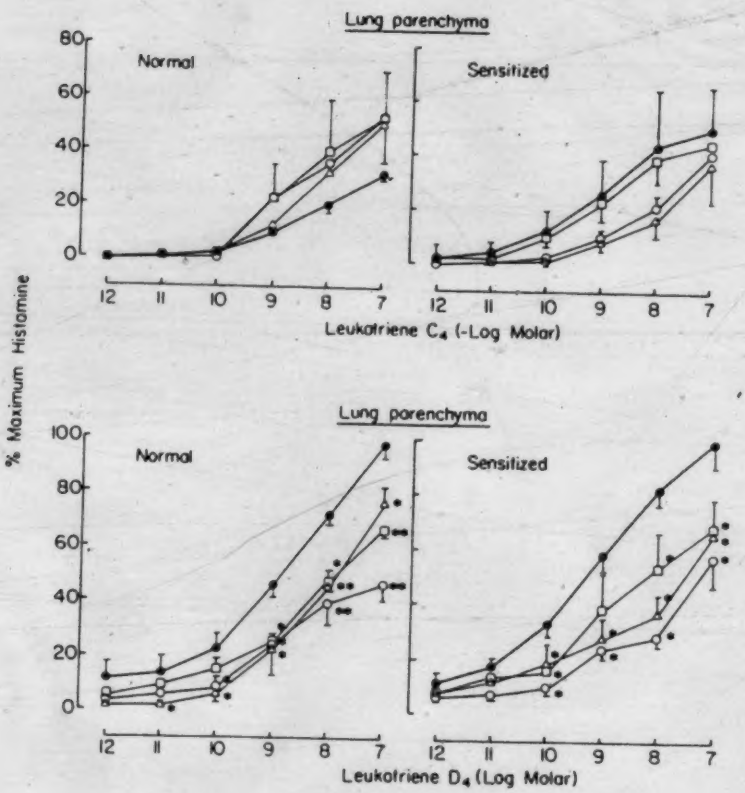


Fig. 10. Concentration-response curves to leukotrienes C_4 (n=3) and D_4 (n=6) on normal and sensitized lung parenchymal strips, after pretreatment with indomethacin (8.5 μM) (□), NDGA (30 μM) (△), phenidone (185 μM) (○), or the paired control tissue (●). The responses to leukotriene C_4 (upper panel) were those obtained after generation of an initial concentration-response curve. Results are mean±SEM. Significant differences from paired control responses are as indicated: *$p<0.05$ and **$p<0.01$.

Table 10. Effect of inhibitors on sensitivity (pD_2) of tracheal spirals to histamine and carbachol.

	Histamine		Carbachol	
Inhibitors	Normal	Sensitized	Normal	Sensitized
Indomethacin (8.5 μM)	5.33±0.13 (8)	5.37±0.08 (12)	6.41±0.08 (9)	6.53±0.09 (9)
Phenidone (185 μM)	5.47±0.16 (8)	5.45±0.12 (9)	6.26±0.08* (8)	6.32±0.05** (8)
NDGA (30 μM)	5.32±0.16 (8)	5.22±0.06 (11)	6.53±0.07 (9)	6.56±0.06 (9)
Time and vehicle control	5.21±0.06 (8)	5.19±0.20 (12)	6.52±0.09 (10)	6.57±0.07 (9)

NOTE: Mean ± SEM, values in parentheses are number of animals.

*$p < 0.05$, **$p<0.01$., relative to control tissue.

was significantly reduced ($p<0.05$) following incubation with phenidone (185 μM). The sensitivity of tracheal spirals and parenchymal strips to U-44069 was unaffected by indomethacin treatment.

4.1.4 Relaxant responses to bronchodilators:

Isoproterenol, VIP, PGE_2 and forskolin caused a dose-dependent relaxation of the guinea-pig trachea and the lung parenchymal strips (Fig. 11). There was no difference between normal and sensitized tissues in the relaxant responses to all four drugs used. The rank order of potency of these agents on the airways was VIP > isoproterenol > PGE_2 > forskolin (Table 11). The parenchyma was more sensitive than the trachea to these drugs. Normal and sensitized tissues exhibited similar sensitivity to these agents. Isoproterenol was the most efficacious drug used on the trachea causing the most relaxation (Fig.11, Table 12). Forskolin was the next most efficacious drug with VIP and PGE_2 being equivalent. PGE_2 relaxed airways at low concentrations. However, at higher concentrations (>1 μM) this agent sometimes contracted the lung parenchymal strip (Fig. 11).

4.1.5 Effects of indomethacin-pretreatment on drug-induced relaxations:

Indomethacin (8.5 μM)-pretreatment did not significantly change

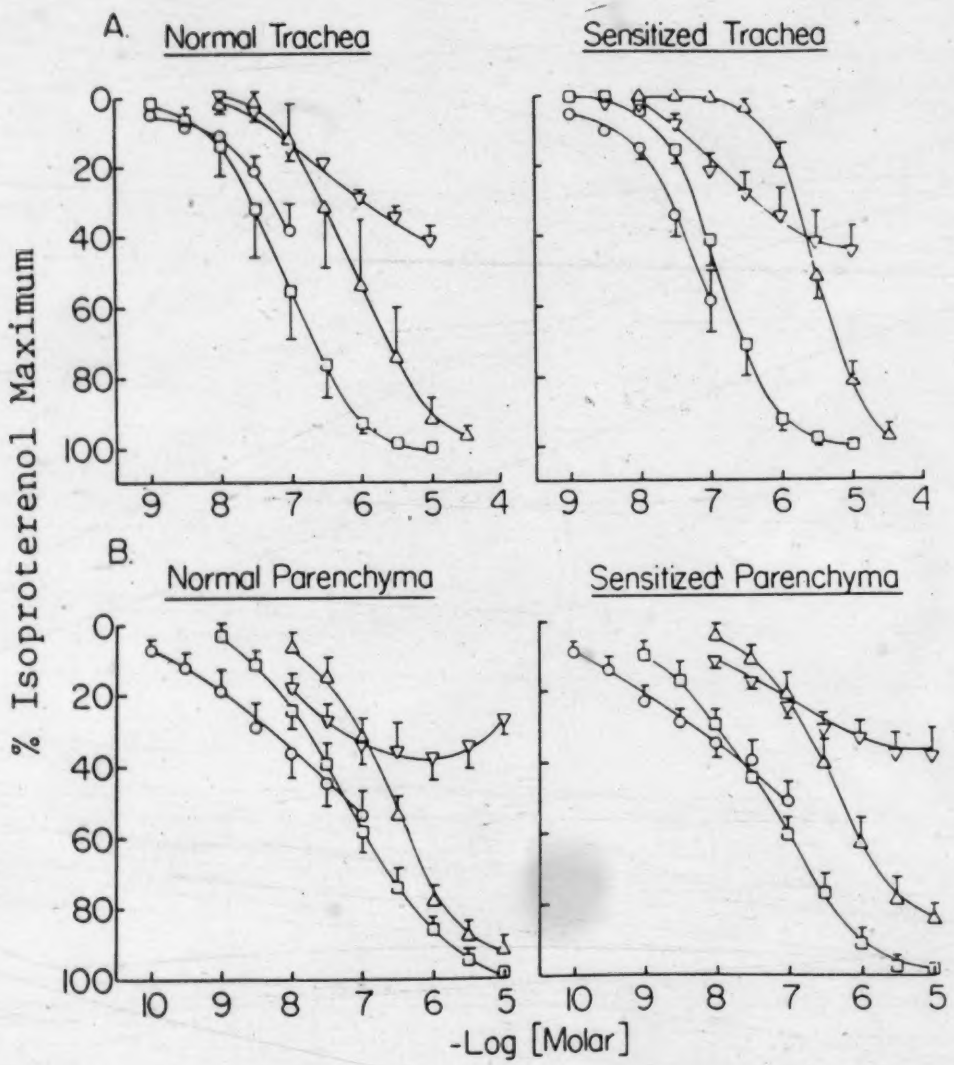


Fig. 11. The relaxant responses of normal and ovalbumin-sensitized guinea-pig tracheal spirals (a) and lung parenchymal strips (b). Symbols indicate the following: (□) isoproterenol, (△) forskolin, (○) vasoactive intestinal peptide and (▽) prostaglandin E_2. Tracheal spirals were precontracted with carbachol (1 μM) and lung parenchymal strips were mechanically adjusted 1 g to baseline tension. Results are mean±SEM (n=5).

Table 11. Sensitivity[a] of airway tissues from normal and sensitized animals to relaxant agents in vitro.

	Trachea		Parenchyma	
Agonist	Normal	Sensitized	Normal	Sensitized
Isoproterenol	7.11±0.27[b]	6.80±0.13	7.19±0.17	7.30±0.11
Forskolin	6.10±0.30	5.44±0.13	6.66±0.09	6.40±0.13
VIP	7.66±0.06	7.59±0.05	8.51±0.18	8.73±0.14
PGE_2	6.54±0.15	6.83±0.28	7.62±0.24	7.37±0.21

[a] = Negative logarithm of the concentration of each drug required to achieve 50% of the maximum response to that drug.

[b]Mean±SEM (n=5).

Table 12. Maximum control relaxations (mg force mg^{-1} tissue dry weight) of airway tissues from normal and ovalbumin-sensitized guinea pigs after addition of relaxant drugs in vitro.

	Trachea		Parenchyma	
Agonist	Normal	Sensitized	Normal	Sensitized
Isoproterenol (10 μM)	120.8±21.4[a]	111.9±18.5	7.9±0.7	9.3±1.1
Forskolin (30 μM)	106.7±17.8	86.8±10.2	10.8±1.4	11.7±0.8
VIP (0.1 μM)	35.7±10.3	69.7±18.8	3.3±0.5	4.6±1.0
PGE_2 (trachea: 30 μM) or (parenchyma: 10 μM)	39.2± 8.3	55.9±16.0	2.6±0.8	3.1±0.7

[a] = Mean±SEM of 5 experiments.

the efficacy of isoproterenol, VIP, PGE_2 or forskolin in causing relaxations of the normal or sensitized trachea (Fig. 12). Furthermore, the sensitivity of the tissues to the drugs (expressed as the negative logarithm of the EC_{50}) was unchanged. All comparisons were relative to the paired time and vehicle control tissue (Table 13).

Indomethacin (8.5 μM)-pretreatment significantly ($p<0.05$) reduced the magnitude of the relaxation of parenchyma induced by high concentrations of isoproterenol (1-10 μM), forkskolin (1-10 μM) and PGE_2 (0.1-10 μM) on normal and sensitized lung parenchyma (Fig. 13). However, the EC_{50} of each drug was unchanged when compared to control. As with the trachea, all comparisons are relative to the paired time and vehicle control tissue (Table 13).

4.1.6 Effects of bronchodilators on adenylate cyclase activity in control and indomethacin-pretreated lung membranes:

The adenylate cyclase of normal and sensitized guinea-pig lung had basal activities (measured in the absence of any drugs) of 54.8± 9.6 and 60.3±8.2 pmol cyclic AMP mg^{-1} protein min^{-1}, respectively. Responses of lung adenylate cyclase to isoproterenol were hormone-specific (Fig. 14) since they were abolished by the β-adrenoceptor antagonist propranolol (1 μM), whereas the responses to PGE_2, VIP and forskolin were unaffected by propranolol. Isoproterenol, VIP, PGE_2 and forskolin stimulated normal and sensitized guinea-pig lung adenylate cyclase activity. There was no difference in the magnitude

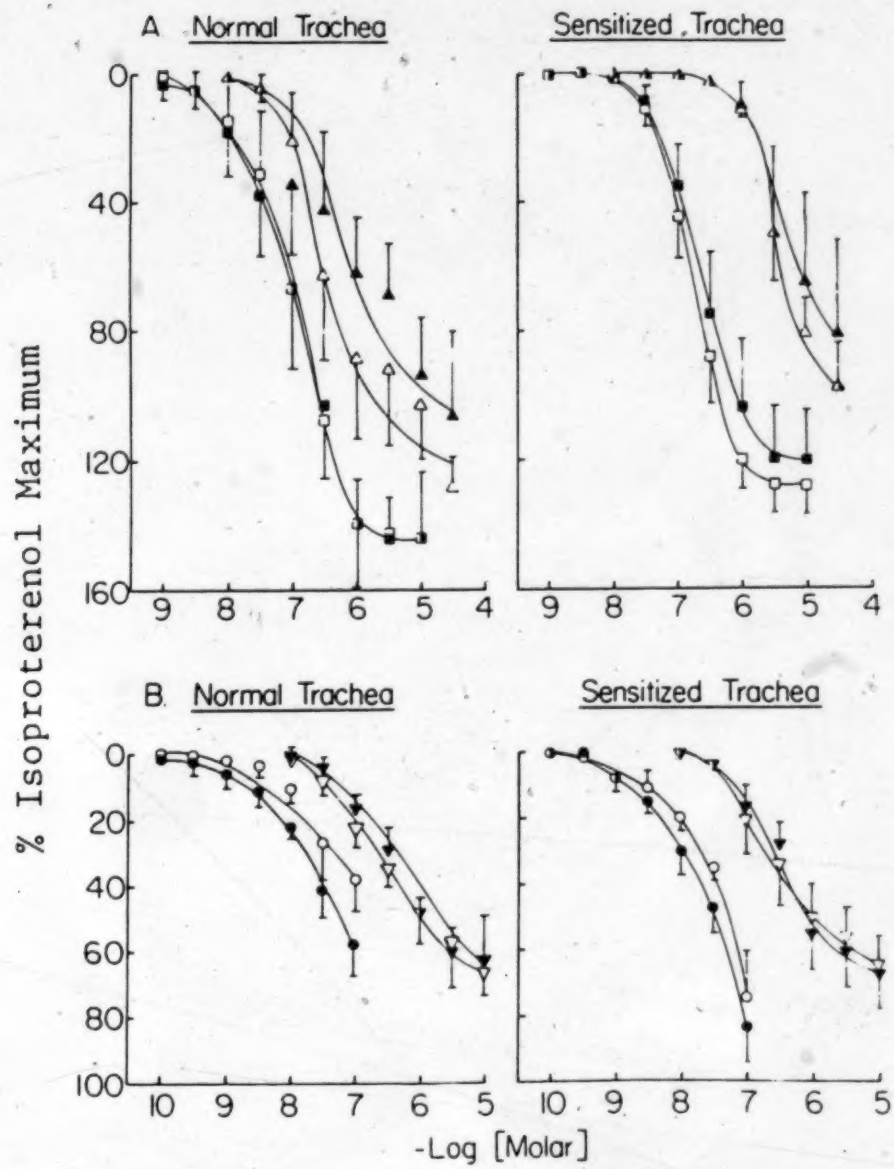


Fig. 12. Effects of indomethacin on the relaxant responses of normal and ovalbumin-sensitized guinea-pig tracheal spirals to isoproterenol (□,■) forkskolin (△,▲) (a) and vascoactive intestinal peptide (○,●), and prostaglandin E_2 (▽,▼) (b). Open symbols represents the responses of the time and vehicle control tissues and the closed symbols represent the responses of the paired indomethacin (8.5 μM)-treated tissues. Results are mean±SEM (n=5).

Table 13. Sensitivity[a] of airway tissue from normal and sensitized animals to relaxant agents in vitro. Effect of indomethacin pretreatment.

	Trachea		Parenchyma	
Agonist	Normal	Sensitized	Normal	Sensitized
Isoproterenol	6.90±2.22[b]	6.80±0.13	7.10±0.20	7.30±0.19
	(7.17±0.37)[c]	(6.60±0.19)	(7.14±0.17)	(7.21±0.17)
Forskolin	6.24±0.5	5.50±0.14	6.26±0.33	6.25±0.20
	(6.10±0.39)	(5.51±0.26)	(6.37±0.29)	(5.81±0.25)
VIP	7.71±0.16	7.51±0.05	8.45±0.26	8.67±0.24
	(7.85±0.14)	(7.65±0.13)	(8.44±0.28)	(8.96±0.49)
PGE_2	6.62±0.24	6.53±0.23	6.78±0.09	7.26±0.19
	(6.40±0.07)	(6.66±0.22)	(not determined)	(not determined)

[a] = -Logarithm of EC_{50}

[b] = Time control tissue

[c] = Indomethacin (8.5 μM) treated.

values are mean±SEM (n=5).

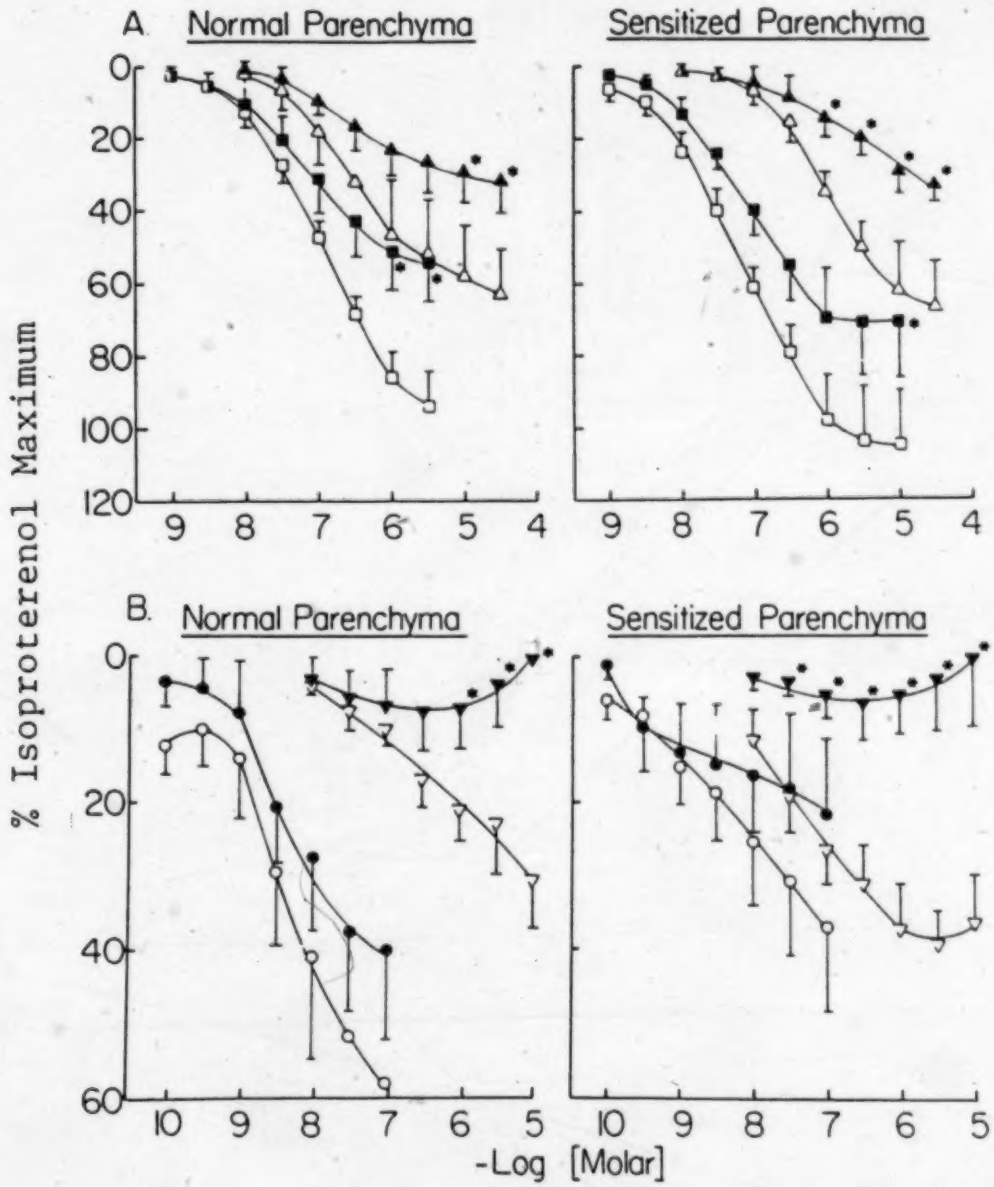


Fig. 13. Effects of indomethacin on the relaxant responses of normal and ovalbumin-sensitized guinea-pig lung parenchymal strips to isoproterenol (□, ■), forskolin (△, ▲) (a) and VIP (○, ●), and PGE_2 (▽, ▼) (b). Open symbols represent the responses of the time and vehicle control tissue and the closed symbols represent the responses of the paired indomethacin (8.5 μM)-treated tissue. *Significantly different ($p<0.05$) from the time and vehicle control tissue responses. Results are mean±SEM (n=5).

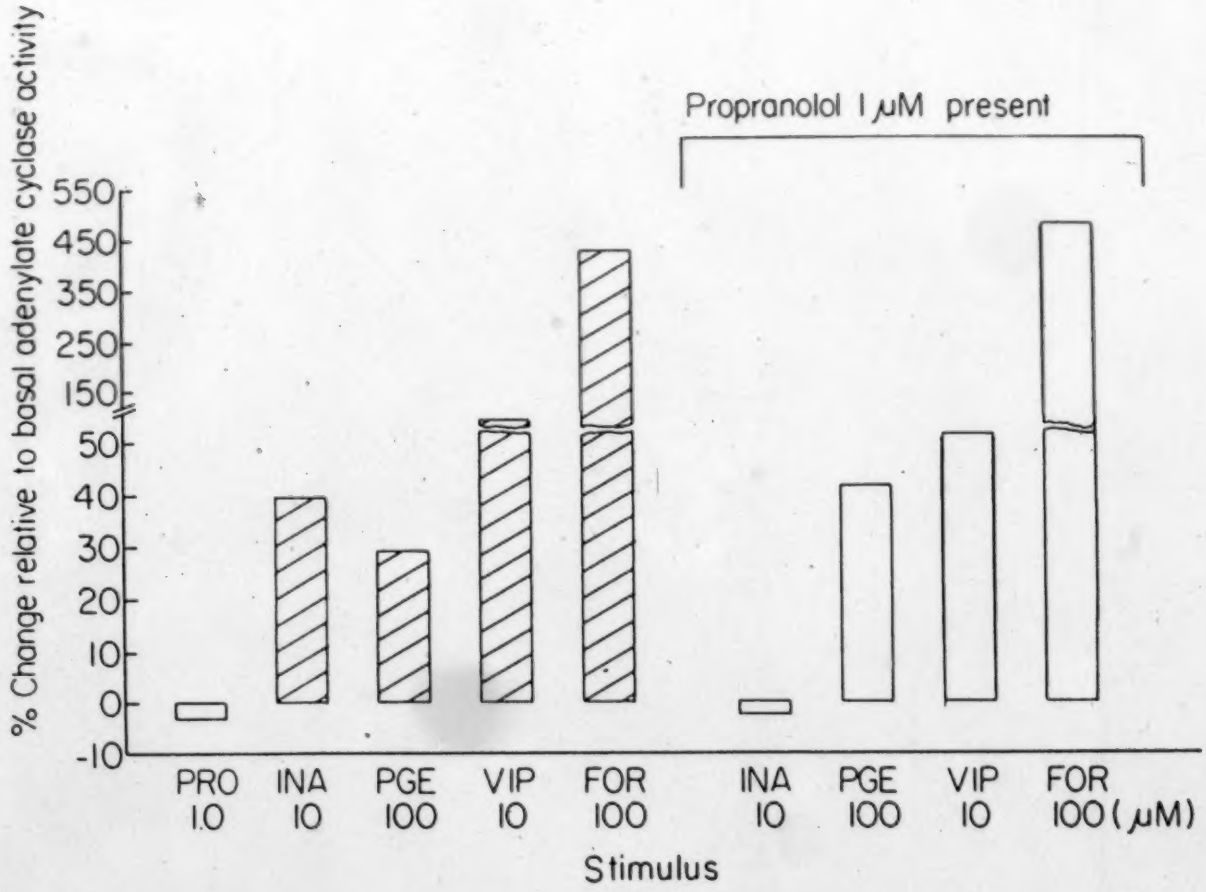


Fig. 14. Hormonal specificity of the responses of guinea-pig lung parenchymal adenylate cyclase. Activation of adenylate cyclase by isoproterenol (INA), prostaglandin E_2 (PGE_2), vasoactive intestinal peptide (VIP) and forskolin (For) in the absence and presence of propranolol (1 μM) is shown. The assays, which were carried out in duplicate, are the mean of results from 2 preparations. Hatched bars are responses in the absence of propranolol and open bars are responses with propranolol (Pro, 1 μM) present.

of the stimulation between normal and sensitized lung (Fig. 15 and 16).

The stimulation of adenylate cyclase by the above drugs was concentration-dependent, and occurred at low concentrations (Fig. 15 and 16). The rank order of efficacy of these drugs on lung adenylate cyclase activation was forskolin>isoproterenol=VIP>PGE_2. Forskolin was the most efficacious drug on lung adenylate cyclase. Basal lung adenylate cyclase activity was increased to 500.0±50.5% of basal activity with a forskolin concentration 100 μM (Fig. 16).

4.1.6.1 Effect of indomethacin-pretreatment on adenylate cyclase activation:

Indomethacin (8.5 μM)-pretreatment of guinea-pig lung parenchyma did not significantly change basal activity or drug-stimulated activity of the enzyme (Fig. 16, Table 14). The effects of indomethacin-pretreatment on the forskolin-induced activation of adenylate cyclase of normal and sensitized lung are shown in Fig. 16.

4.2 ANTIGEN-, A23187-, AND AA-INDUCED CONTRACTIONS OF NORMAL AND SENSITIZED AIRWAY TISSUES.

4.2.1 Effects of indomethacin, NDGA, or mepacrine:

A23187 contracted normal and sensitized tissues in a concentration-dependent fashion (Figs. 17 and 18). There was no difference in the magnitude of the induced contraction between normal

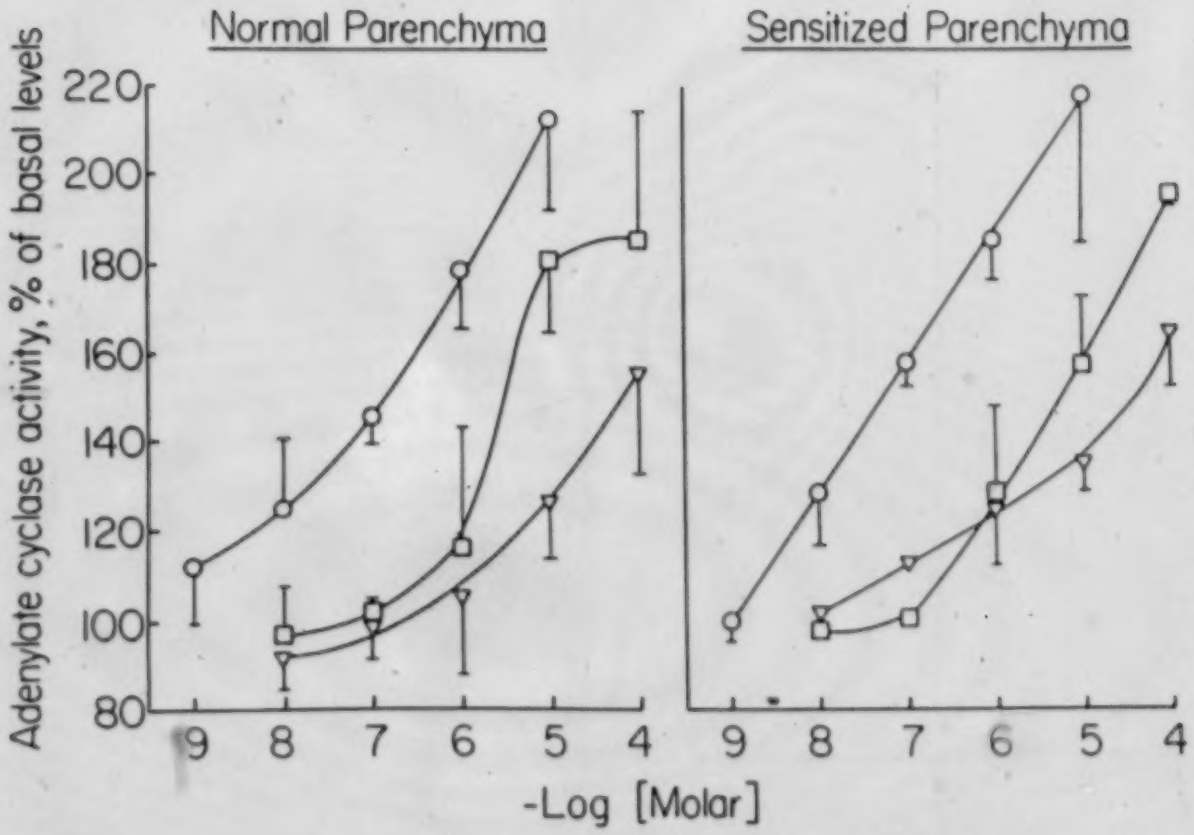


Fig. 15. Activation of normal (a) and ovalbumin-sensitized (b) guinea-pig lung adenylate cyclase by isoproterenol (□), VIP (○) and PGE_2 (▽). Assays were carried out in duplicate and the results are the mean±SEM of experiments using 4 different preparations each of normal and sensitized lung. Adenylate cyclase activity is expressed as a percentage of the basal activity measured in the absence of any drug.

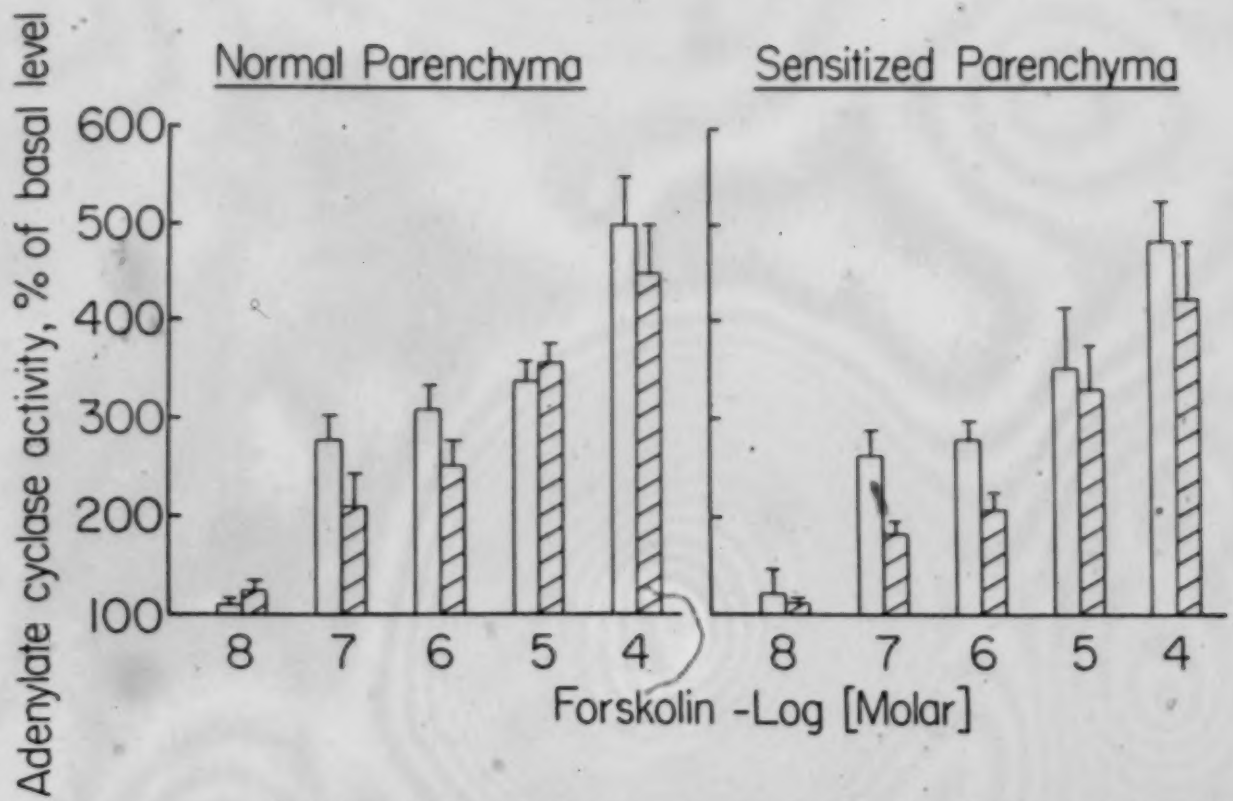


Fig. 16. Effects of indomethacin (8.5 μM)-pretreatment on normal (a) and sensitizied (b) guinea-pig lung adenylate cyclase activation induced by forskolin. Open bars are responses without, and hatched bars are responses after indomethacin (8.5 μM)-pretreatment. Assays were carried out in duplicate and the results are the mean±SEM of experiments using 3 different preparations each of normal and sensitized lung. Adenylate cyclase activity is expressed as a percentage of the basal activity measured in the absence of any drug.

Table 14. Adenylate cyclase activity of control and indomethacin (8.5 μM)-pretreated lung membranes. Results are mean±SEM of 4 preparations.

	Normal Lung		Sensitized lung	
	Control	Indomethacin	Control	Indomethacin
Basal activity (Pmoles mg^{-1} min^{-1})	54.8± 9.6	41.8± 3.8	62.3± 9.3	50.5± 7.2
% of basal activity				
Isoproterenol (10 μM)	181.1±16.6	180.6±16.1	158.4±15.3	157.7±15.8
PGE_2 (100 μM)	156.6±21.9	146.1±16.4	165.1±11.6	156.9± 6.1
VIP (1 μM)	179.0±13.5	180.0±27.7	186.3± 9.6	171.7±18.5

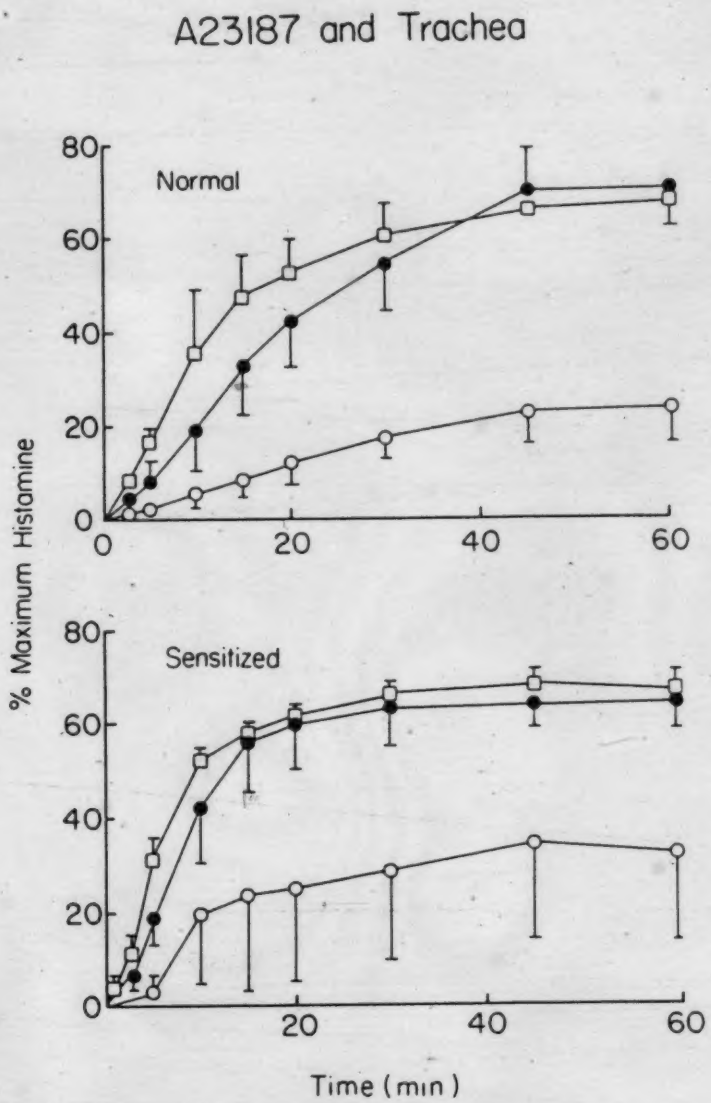


Fig. 17. Contraction of normal and sensitized trachea by A23187 at 0.19 μM (○), 1.9 μM (●), and 5.7 μM (□). Responses are expressed as a percentage of the maximum response to histamine (100 μM) obtained initially. Results are mean±SEM (n=5).

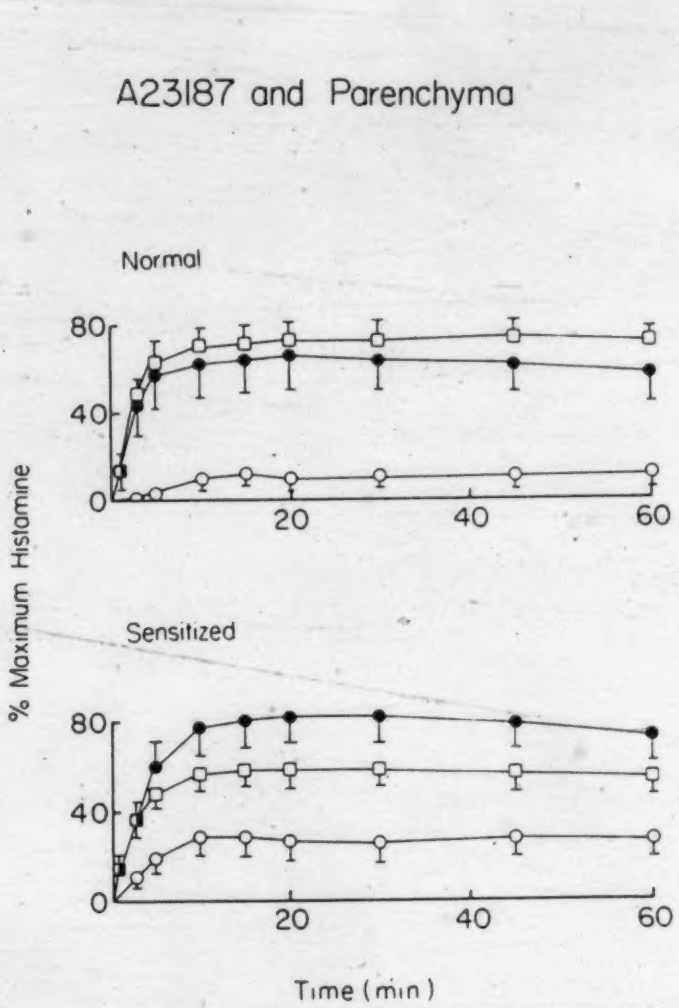


Fig. 18. Contraction of normal and sensitized lung parenchymal strips by A23187 at 0.19 μM (○), 1.9 μM (●), and 5.7 μM (□). Responses are expressed as percentage of the maximum response to histamine (100 μM) obtained initially. Results are mean±SEM (n=5).

and sensitized tissues (trachea or parenchyma). The contractions induced by A23187 were prolonged and the profile of contractions were different from those induced by OA (Fig. 19). The maximal contractile responses induced by A23187 or OA were at 5.7 μM and 3 μg/ml, respectively. These concentrations were used in further experiments.

Indomethacin (8.5 μM)-pretreatment enhanced both types of contractions significantly ($p<0.05$) (Fig. 20). The presence of AA (32.8 μM) did not further alter the responses of indomethacin pretreated tissues to either OA or A23187. This concentration of AA contracts the tissues on its own to about 30% of maximal histamine (data not shown).

NDGA (50 μM) pretreatment either completely abolished or reduced OA- and A23187-induced contractions of the trachea (Fig. 20).

AA slightly relaxed tracheal spirals and contracted lung parenchymal strips before indomethacin-pretreated. AA (66 μM)-induced contractions after indomethacin-pretreatment were unaffected by mepacrine (210 μM) (a phospholipase A_2 inhibitor)-pretreatment of normal and sensitized trachea (Fig. 21) and parenchyma (Fig. 22). The extent of contraction to AA (66 μM) after indomethacin-pretreatment is shown for normal and sensitized trachea (Fig. 23) and for normal and sensitized lung parenchyma (Fig. 24). The trachea (93.3±20.1% histamine maximum) was more responsive to AA than the lung strip (32.5±5.1% histamine maximum), contracting to a greater extent with respect to the maximal contraction obtained with histamine. This concentration of AA induces approximately 80% of the respective maximum

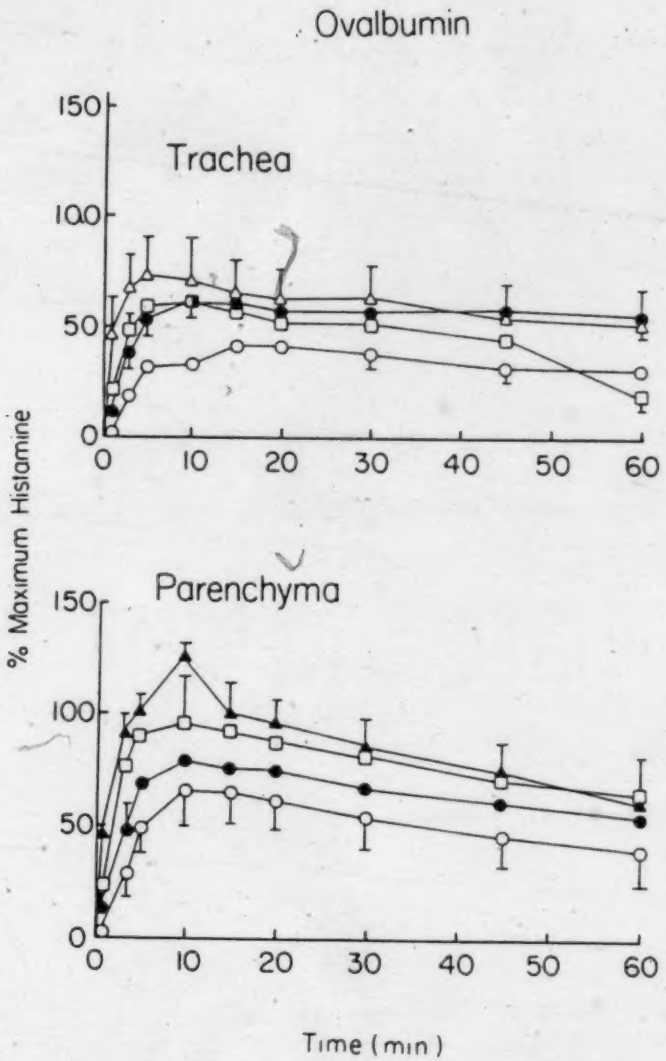


Fig. 19. Responses of sensitized airway tissue to ovalbumin 0.1 μg/ml (○), 1.0 μg/ml (●), 3.0 μg/ml (□), and 10 μg/ml (▲). Responses are expressed as a % of the maximum response to histamine (100 μM) obtained initially. Results are mean±SEM (n=5).

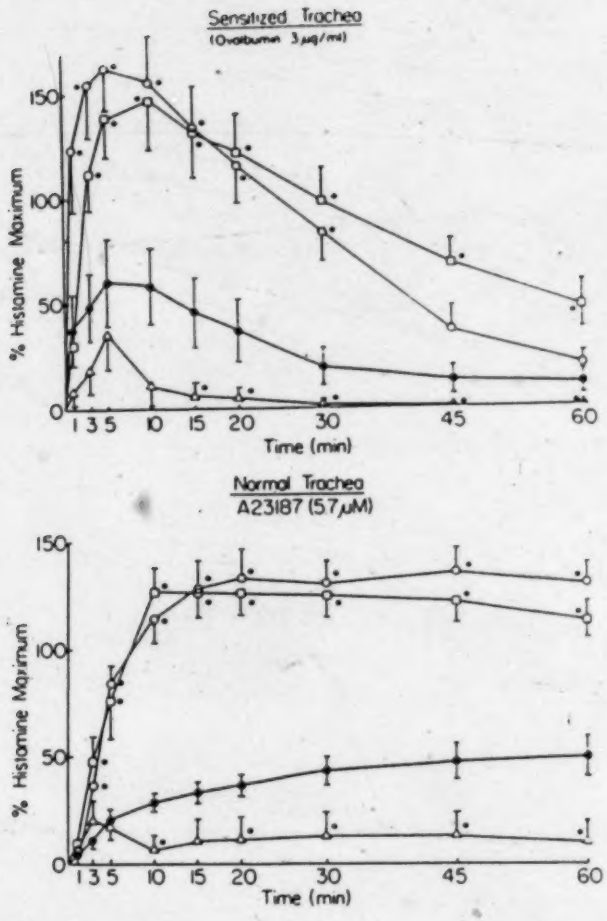


Fig. 20. Contractile response of normal and sensitized trachea to A23187 and ovalbumin. Control responses (●), indomethacin (8.5 μM) pretreated tissues (○), indomethacin and arachidonic acid (32.8 μM) (□) and nordihydroguaiaretic acid (50 μM) pretreated tissues (△). Values are mean ± SEM (n=6 for each curve). Statistical significance was assessed by comparison with the appropriate control and are as indicated: *$p<0.05$.

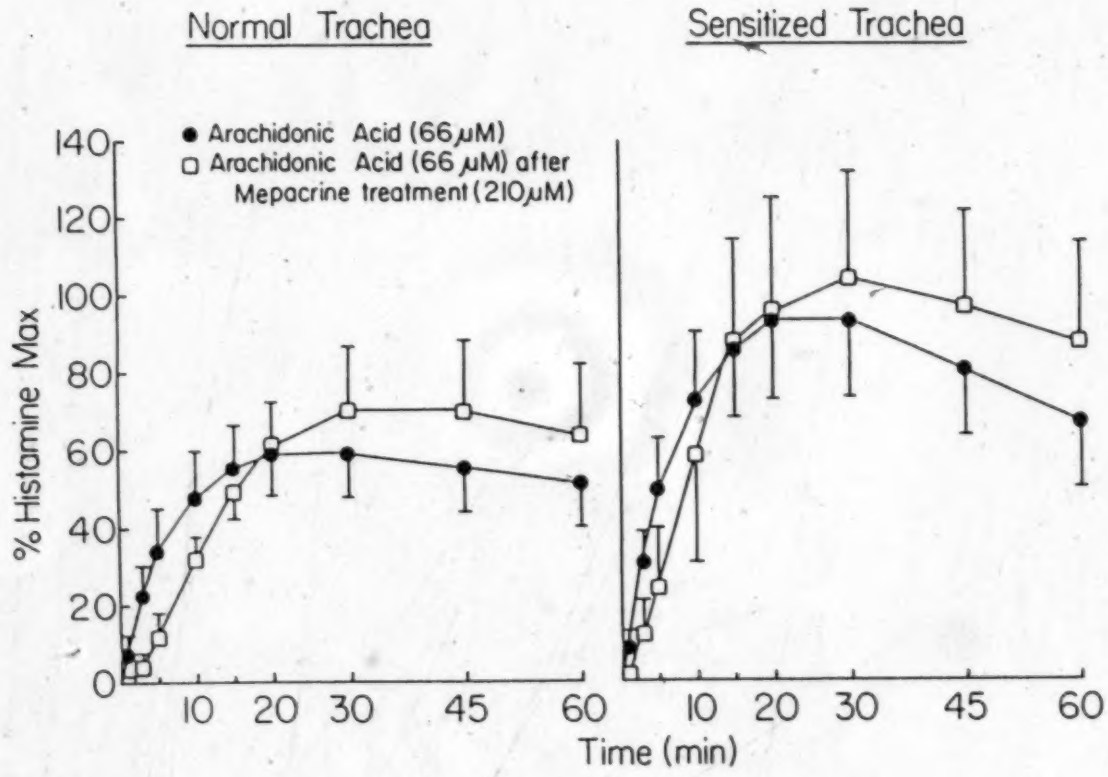


Fig. 21. The responses of normal and sensitized trachea to AA (66 μM). Tissues were pretreated with indomethacin (8.5 μM). The effects of mepacrine (210 μM)-pretreatment are shown. Results are mean±SEM (n=5).

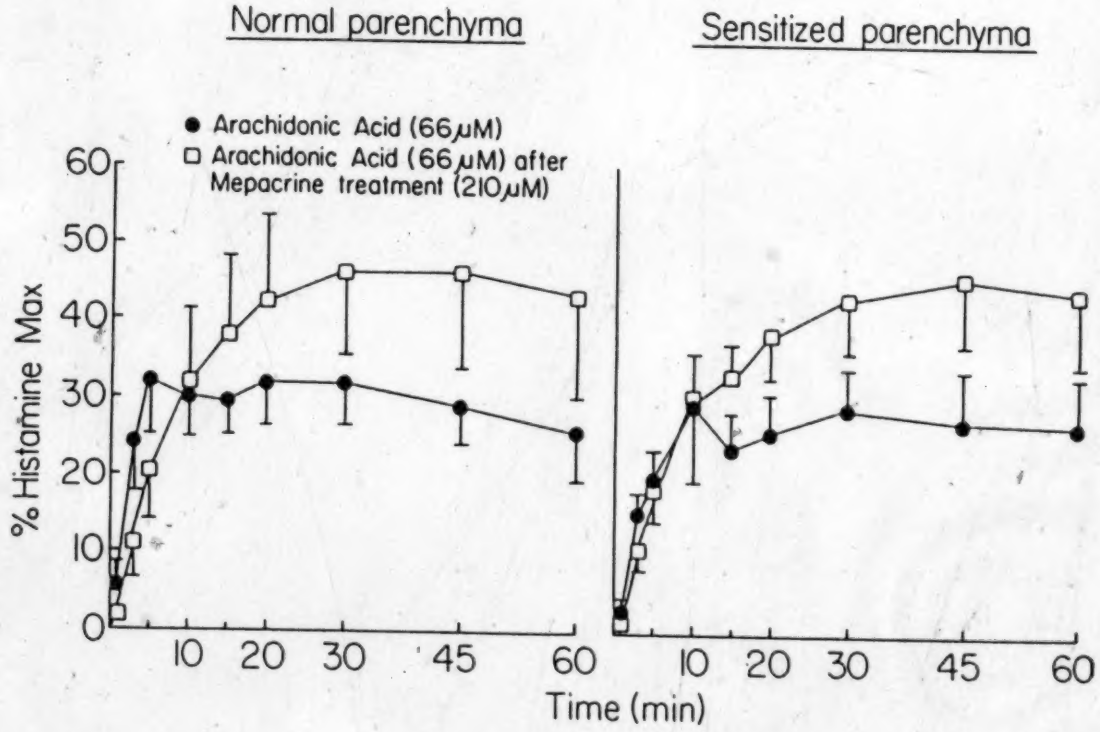


Fig. 22. The responses of normal and sensitized parenchymal strips to AA (66 μM). Tissues were pretreated with indomethacin (8.5 μM). The effects of mepacrine (210 μM)-pretreatment are shown. Results are mean±SEM (n=5).

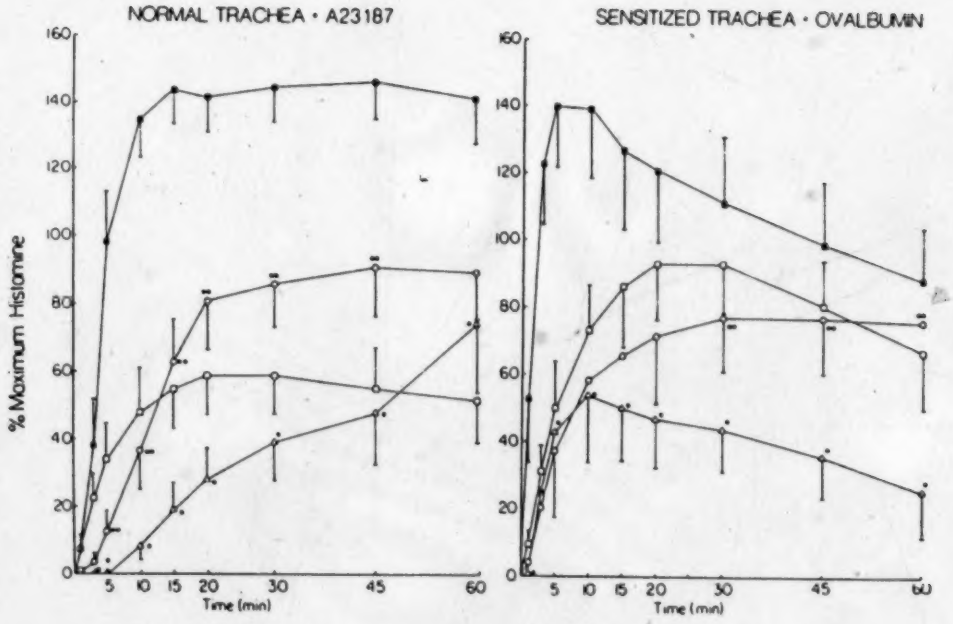


Fig. 23. The contractile responses of indomethacin-treated tracheal spirals to AA, A23187 or OA: (a) normal trachea plus A23187: (b) sensitized trachea plus OA. Results are the mean±SEM of at least 5 experiments. Symbols indicate the following: (□) AA alone; (■) A23187 or OA alone; (△) mepacrine and A23187 or mepacrine and OA; and (○) mepacrine AA and A23187 or mepacrine, AA and OA. *Significantly different ($p<0.05$) from contractions to A23187 (a) or OA alone (b) indicating that mepacrine partly inhibits contractions induced by A23187 or OA; **significantly different ($p<0.05$) from contractions to mepacrine-treated tissues stimulated with A23187 (a) or OA alone (b), indicating that addition of exogenous substrate (i.e. AA) enhanced contractions previously inhibited by mepacrine.

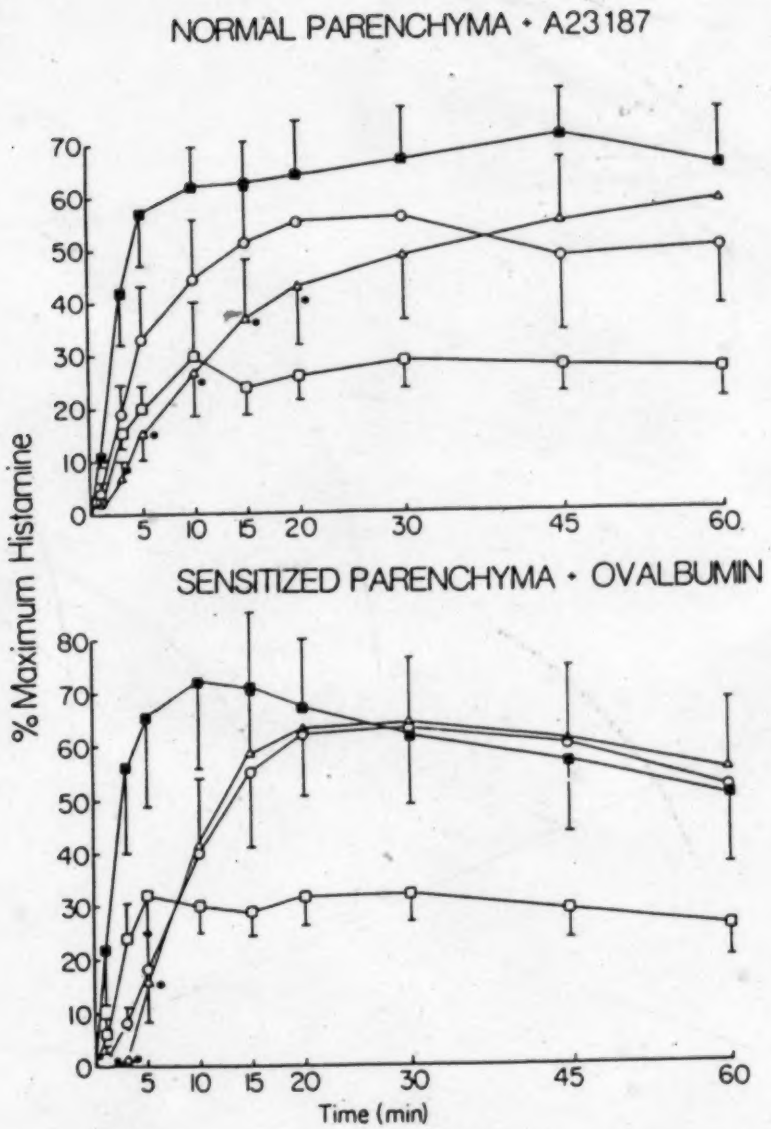


Fig. 24. The contractile responses of indomethacin-treated lung parenchymal strips to AA, A23187 or OA; (a) normal parenchyma plus A23187, (b) sensitized parenchymal plus OA. Results are the mean±SEM of at least 5 experiments. Symbols indicate the following; (□) AA alone; (■) A23187 or OA alone; (△) mepacrine and A23187 or mepacrine and ovalbumin; and (○) mepacrine, AA and A23187 or mepacrine, AA and OA. *Significantly different ($p<0.05$) from contractions to A23187 or OA alone, indicating that mepacrine partly inhibits contractions induced by A23187 or OA.

effect possible on trachea or parenchyma. There was no statistically significant difference in the extent and magnitude of AA-induced contractions between normal and sensitized tissues (Figs. 23 and 24).

NDGA (100 μM), a lipoxygenase inhibitor, significantly ($p<0.05$) reduced the magnitude of the contraction at most time points studied (Figs. 25 and 26). Phenidone (185 μM), another lipoxygenase inhibitor, exhibited similar effects (2 experiments). A23187-induced contractions (normal trachea) and OA-induced contractions (sensitized trachea) were significantly reduced) ($p<0.05$) after mepacrine pretreatment (Fig. 23). Addition of exogenous AA to mepacrine-pretreated trachea increased the magnitude of the contraction to A23187 or OA after (mepacrine-pretreatment), but not to the level observed in the absence of mepacrine (Fig. 23). Mepacrine pretreatment significantly reduced the extent of contraction of lung parenchyma to A23187 or OA in the initial period (0-10 min) after stimulus addition. Exogenous AA did not further increase the magnitude of the contractions (Fig. 24).

NDGA (100 μM) pretreatment of airway tissues markedly reduced the extent of contraction to AA and A23187 or AA and OA (normal or sensitized tissues) (Figs. 25 and 26).

4.2.2 High performance liquid chromatography:

Analysis of the material initially purified with SEP-PAKs by HPLC indicated the presence of peaks cochromatographing with synthetic LTC_4 and LTD_4 (Fig. 27). The retention times for these substances were 5.27

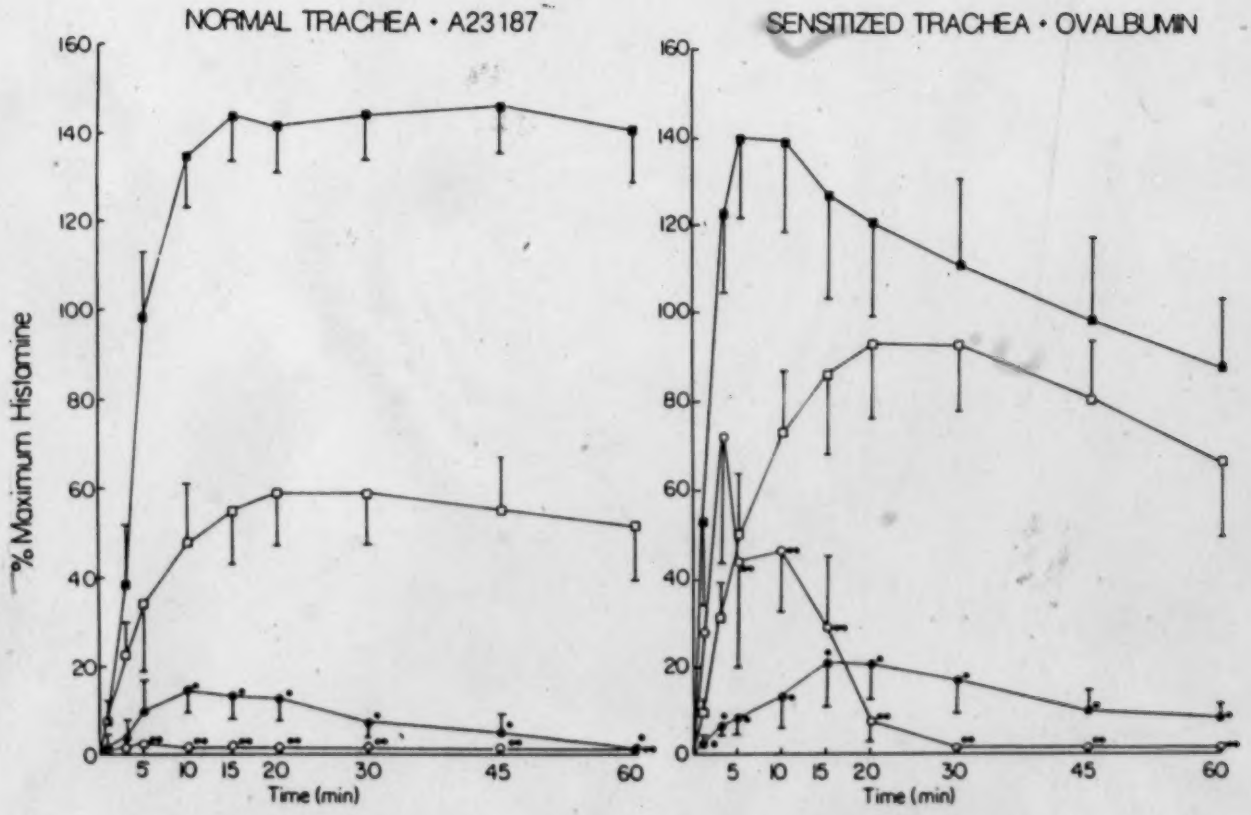


Fig. 25. The contractile responses of indomethacin-treated tracheal spirals to or AA, A23187 or OA (a) normal trachea plus A23187; (b) sensitized trachea plus OA. Results are mean±SEM. Symbols indicate the following: (□) AA alone (n=8); (■) A23187 or ovalbumin alone (n=5); (●) nordihydroguaiaretic acid (NDGA) and AA (n=5); and (○) NDGA and A23187 and AA or OA and AA (n=3). *Significantly different ($p<0.05$) from contractions to AA alone and **significantly different ($p<0.05$) from contractions to A23187 of AA alone.

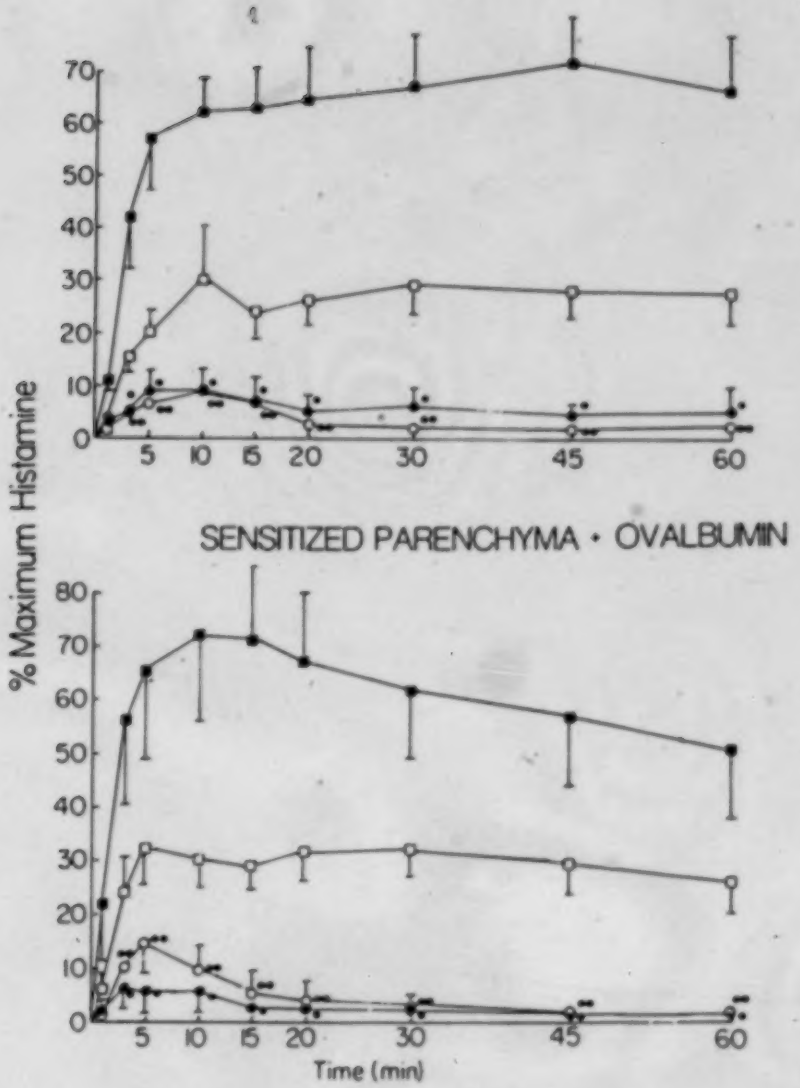


Fig. 26. The contractile responses of indomethacin-treated lung parenchymal strips to AA, A23187 or OA: (a) normal parenchyma plus A23187; (b) sensitized parenchyma plus OA. Results are mean±SEM. Symbols indicate the following; (□) AA alone (n=6); (■) A23187 or ovalbumin alone (n=5); (●) nordihydroguaiaretic acid (NDGA) and AA (n=5); and (○) NDGA and A23187 and AA or OA and AA (n=3). *Significantly different ($p<0.05$) from contractions to AA alone and **significantly different ($p<0.05$) from contractions to A23187 or ovalbumin alone.

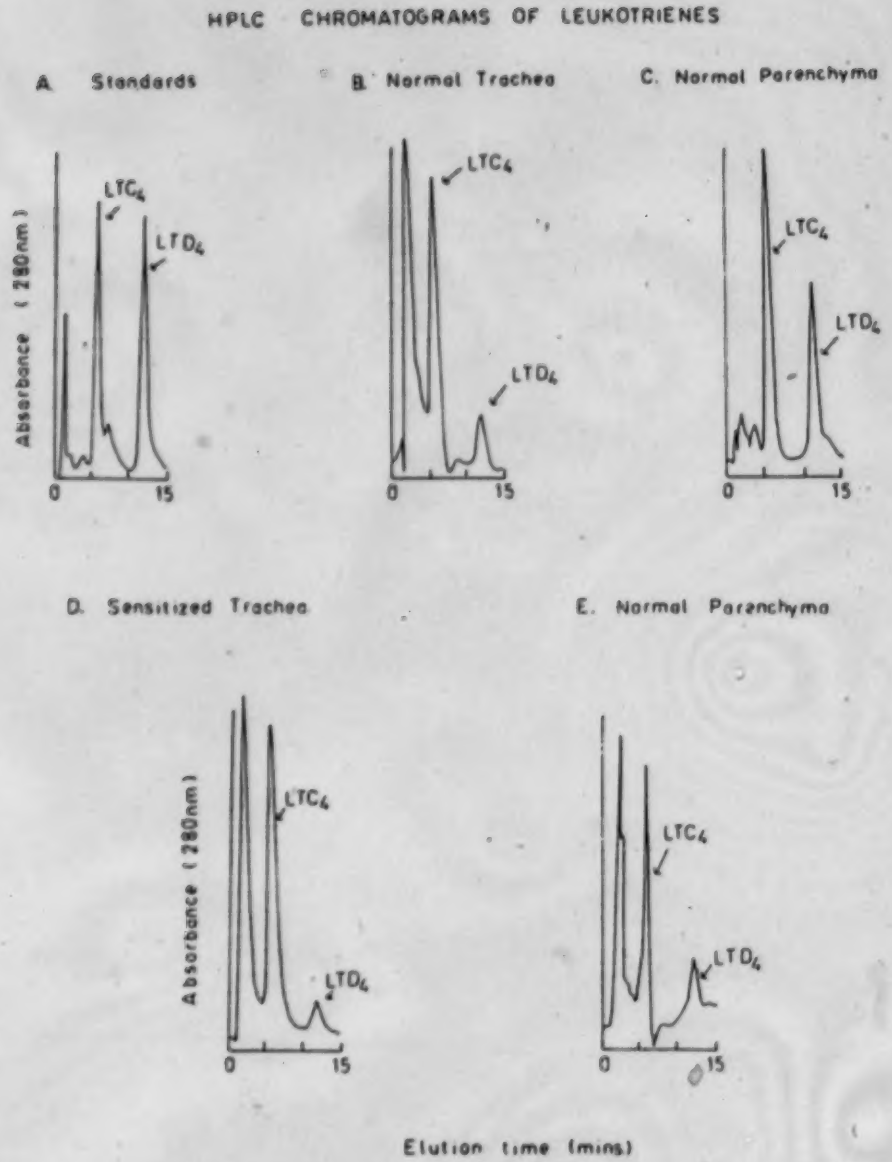


Fig. 27. Typical HPLC chromatograms obtained after analysis of the medium surrounding airway tissues after appropriate stimulation. Synthetic standards LTC_4 and LTD_4 are shown in (A); Normal trachea after stimulation with AA in (B); Normal parenchyma after AA stimulation in (C); sensitized trachea after mepacrine treatment and AA stimulation with OA in (D); and normal parenchyma after A23187 in (E). All absorbances were measured at 280 nm.

and 11.50 min, respectively. The peaks had an ultraviolet absorption spectrum with a maximum at 278-280 nm with typical shoulders (Fig. 28) characteristic of the the conjugated triene structure of LTs. Numerous samples were subjected to HPLC and the results shown in Figs.27 and 28 are representative.

4.2.3 Bioassay of released LTs:

The crude SEP-PAK extracts of the bath fluid, and the chromatographed samples that coincided with either LTC_4 or LTD_4 in retention time, exhibited spasmogenic activity on guinea-pig ileum and lung parenchymal strips. Both contractile activities were prevented or reversed by the LT antagonist FPL55712 (Fig. 29).

From the HPLC studies, it was noted that LTC_4 was the predominant mediator released from the airways (LTC_4:LTD_4=20:1 ratio) using AA and as the stimulus. This is based on % composition of samples determined by integration of the area under the HPLC peaks. We used synthetic LTC_4 as the standard for bioassay on longitudinal strips of guinea pig ileum. Similar amounts of LTs were released from normal and sensitized tissues under the same conditions. NDGA pretreatment completely abolished or markedly reduced release of LT-like activity (Table 15). Samples obtained from mepacrine-treated tissues could not be bioassayed because the mepacrine residue after initial sample purification relaxed the ileum. Hence the data were considered unreliable. It can be seen from Table 15 that indomethacin-pretreatment reduced the

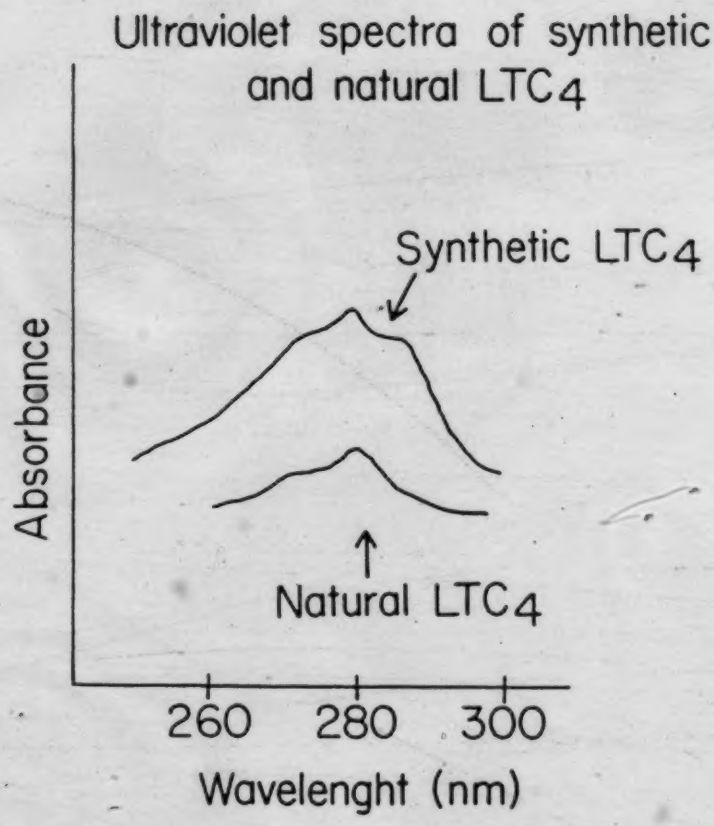


Fig. 28. Ultraviolet spectra of synthetic and natural LTC_4 (released from trachea) recorded in methanol.

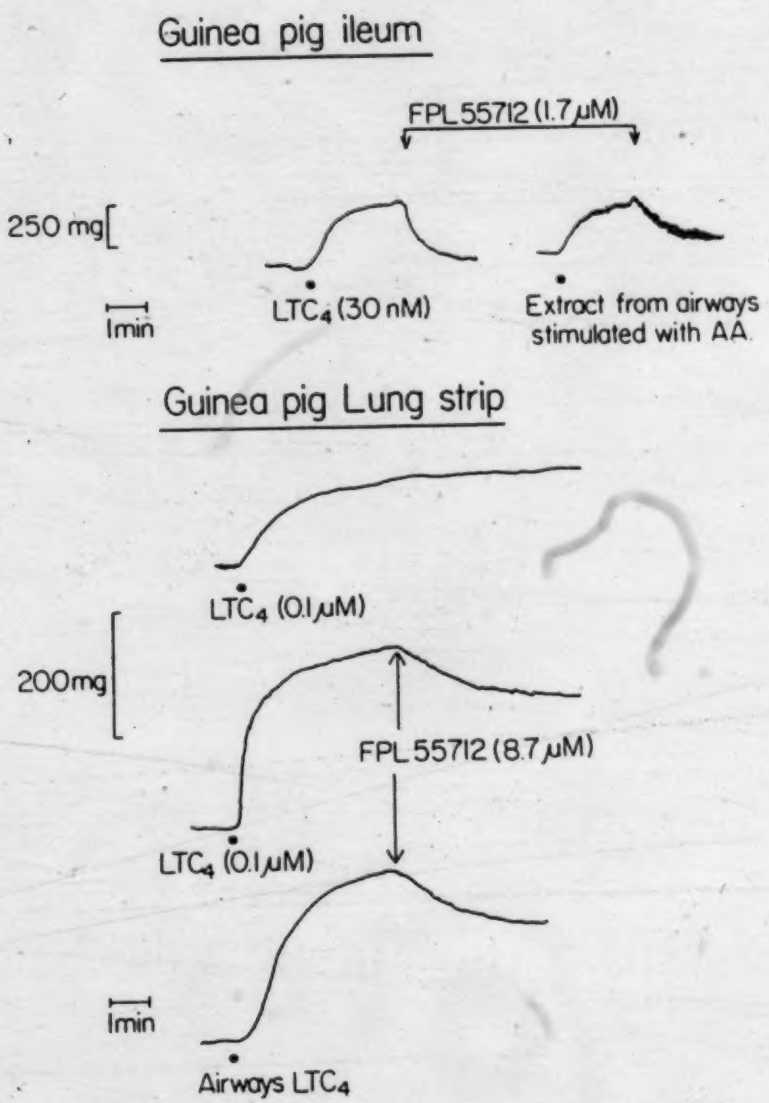


Fig. 29. Bioassay of released LTC_4 on guinea-pig ileum (a) and lung parenchymal strips (b). Synthetic LTC_4 was used as a standard. FPL55712 (1.7 μM) was used to reverse contractions on the ileum. A higher concentration of FPL55712 was necessary to partly reverse contractions on the lung strip. The bioassay was carried out in the presence of atropine (1 μM) and mepyramine (1 μM).

Table 15. Release of LTs from guinea-pig airway tissue (ng/mg tissue [dry weight]) in the first hour. (SEP-PAK Extracted only)

Stimuli	Trachea		Parenchyma	
	Normal	Sensitized	Normal	Sensitized
A23187 (5.7 μM) or OA (3 μg/ml)	90.0±38[a] (8)[b] (2.3 - 274)[c]	84.0±7.20 (7)	N.D.	N.D.
A23187 or OA after indomethacin (8.5 μM)	21.0±17 (5) (0.1 - 89)	20.0±19.0 (4) (0.8 - 77)	N.D.	N.D.
A23187 or OA after indomethacin in the presence of AA (33 μM)	17.0±11 (10) (0.4 - 110)	13.0±9.89 (6) (2.3 - 60.8)	N.D.	N.D.
A23187 or OA after NDGA (50 μM)	5.0±3.0 (5) (0 - 14.5)	0.7±0.6 (9) (0 - 2.6)	N.D.	N.D.
AA (66 μM) after indomethacin (8.5 μM)	21.6±8.8 (13) (1.8 - 124)	33.9±12.9 (9) (4.4 - 129)	11.3±3.5 (8) (0.8 - 34)	12.7±3.5 (1.4 - 21)
AA (66 μM) after NDGA (50 μM)	3.1±2.2 (9) (0 - 20.4)	1.1±0.7 (8) (0 - 5.7)	4.1±1.2 (7) (0 - 8.9)	0.2±0.1 (6) (0 - 0.6)

a = MEAN±SEM
b = Number of animals
c = Range
N.D. = not done

amounts of assayable LTs released by trachea after challenge with A23187 or OA.

4.2.4 Nature of the mediators released from airway tissue after stimulation with AA, A23187 or OA.

From the above studies utilizing effects of lipoxygenase inhibitors, HPLC, and bioassay, the conclusion was reached that stimulation of airway tissue with AA, A23187 or OA (sensitized tissue) resulted in the synthesis of lipoxygenase products of AA, namely LTC_4 and LTD_4.

4.3 ROLE OF CALCIUM IN ARACHIDONIC ACID-INDUCED CONTRACTION OF GUINEA PIG AIRWAYS.

4.3.1 Effects of calcium and TMB-8:

Since we observed that normal and sensitized airway tissues did not differ in their ability to contract to bronchoconstrictors or to relax to bronchodilators, or to synthetize AA-derived metabolites (see 4.1 and 4.2), the following studies of the role of Ca^{2+} in AA-induced contractions were done in normal airway tissue only.

AA (66 μM) contracted the indomethacin-pretreated guinea pig tracheal spiral and parenchymal strip (Fig. 30). The extent of the tracheal contractions in normal KHS was greater than the parenchymal contraction when expressed as a percentage of the histamine maximum. At 20 min trachea contracted to 82.1±9.0% of the histamine maximum, whereas parenchyma contracted to 22.5±6.5% of the histamine maximum.

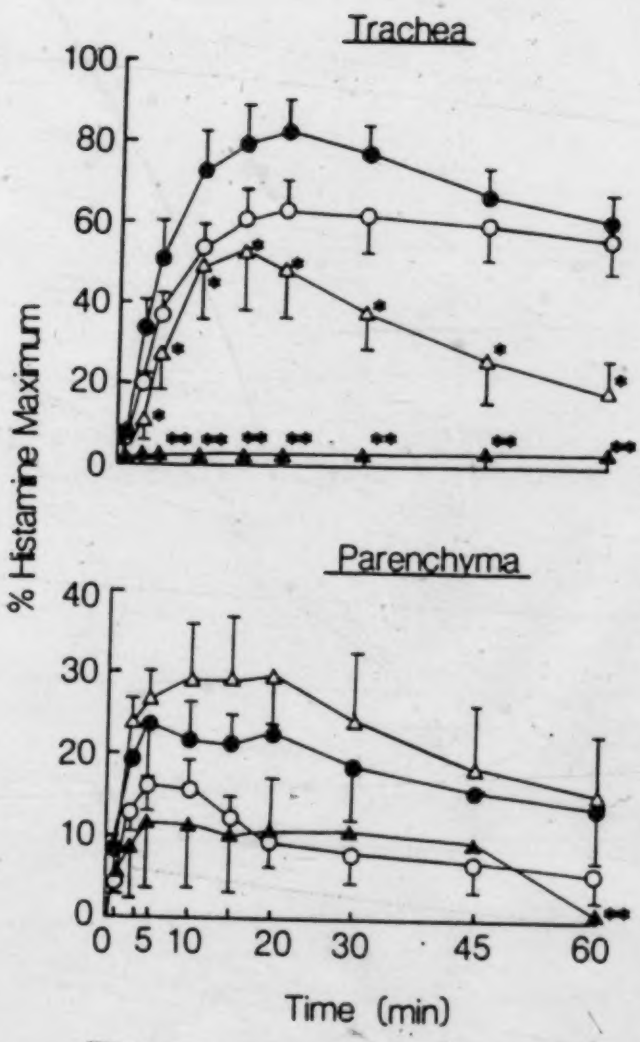


Fig. 30. The effects of calcium and TMB-8 on AA-induced contractions of trachea and parenchyma. The symbols indicate the following: (●) Ca^{2+} (2.2 mM); (△) Ca^{2+}-free (○); Ca^{2+} (8.8 mM); and (▲) Ca^{2+}-free plus TMB-8 (100 μM). *Indicates a significant difference from control ($p<0.05$). **Indicates a significant difference from contractions obtained in Ca^{2+}-free KHS ($p<0.05$). Results are the means±SEM (n=5).

The AA-induced contraction of the trachea in the presence of KHS containing Ca^{2+} (8.8 mM) was not significantly different from the contraction observed in normal KHS. However, after 30 min in Ca^{2+}-free KHS, the tracheal response to AA was significantly reduced ($p<0.05$) at 3-60 min from control (Fig. 30). However, the response was not completely abolished. The response (as % of histamine maximum) at 20 min in Ca^{2+}-free KHS was 48.2±10.0% whereas at the same time time point in normal KHS the response was 82.1±9.0%. The intracellular calcium antagonist, TMB-8 (100 μM), completely abolished the response of trachea to AA in Ca^{2+}-free KHS.

On the parenchyma, AA-induced contractions in Ca^{2+}-free KHS were not significantly different from contractions in normal KHS. In fact, the contractions in Ca^{2+}-free KHS were slightly enhanced (Fig. 30). In KHS containing Ca^{2+} (8.8 mM) there was a slight, but not significant reduction of the response to AA (Fig. 30). TMB-8 reduced the magnitude of the contraction to AA in Ca^{2+}-free KHS. However, this was not significantly different from contractions in Ca^{2+}-free KHS until 60 min following addition of AA. Addition of calcium (2.2 mM) to trachea and parenchyma stimulated with AA in calcium-free KHS in the presence of TMB-8 did not further increase the response of these tissues (data not shown).

4.3.2. Effects of nitrendipine and verapamil:

The contractions of trachea and parenchyma to AA (66 μM) were

expressed as areas under the plotted curve (0-60 min). The areas were calculated using an integrator programme on the University of Alberta computer system. The results are shown as histograms in Fig. 31. Nitrendipine (1, 10 and 100 μM), a calcium entry blocker, caused a dose dependent reduction of the AA-induced contraction of trachea in normal KHS. Verapamil (100 μM) caused a five-fold reduction in the response of the trachea and parenchyma to AA (Fig. 31). Addition of Ca^{2+} (2.2 μM) to make a total Ca^{2+} concentration of 4.4 mM, at the end of 60 min of contraction, did not further increase the response.

4.3.3 Effects of lanthanum and EDTA. Reversal by calcium-readdition:

Lanthanum chloride (1 mM) did not affect AA-induced contractions of trachea and parenchyma in normal KHS (Figs. 32 and 33). However, in Ca^{2+}-free KHS, lanthanum chloride (1 mM) or EDTA (300 μM) completely abolished AA-induced contractions of trachea and significantly ($p<0.05$) reduced parenchymal contractions (Figs. 32 and 33). Addition of Ca^{2+} (2.2 mM) to trachea and parenchyma in Ca^{2+}-free KHS in the presence of lanthanum chloride (1 mM) or EDTA (300 μM) 60 min after stimulation with AA caused a rapidly developing contraction (Figs. 32 and 33). Similar results were obtained when calcium Ca^{2+} (2.2 mM) was added to trachea or parenchyma in Ca^{2+}-free KHS 60 min after stimulation with AA (Figs. 32 and 33). The contractile response obtained after addition of Ca^{2+} was of similar magnitude and time course as that obtained for tissues stimulated in normal KHS. However, on

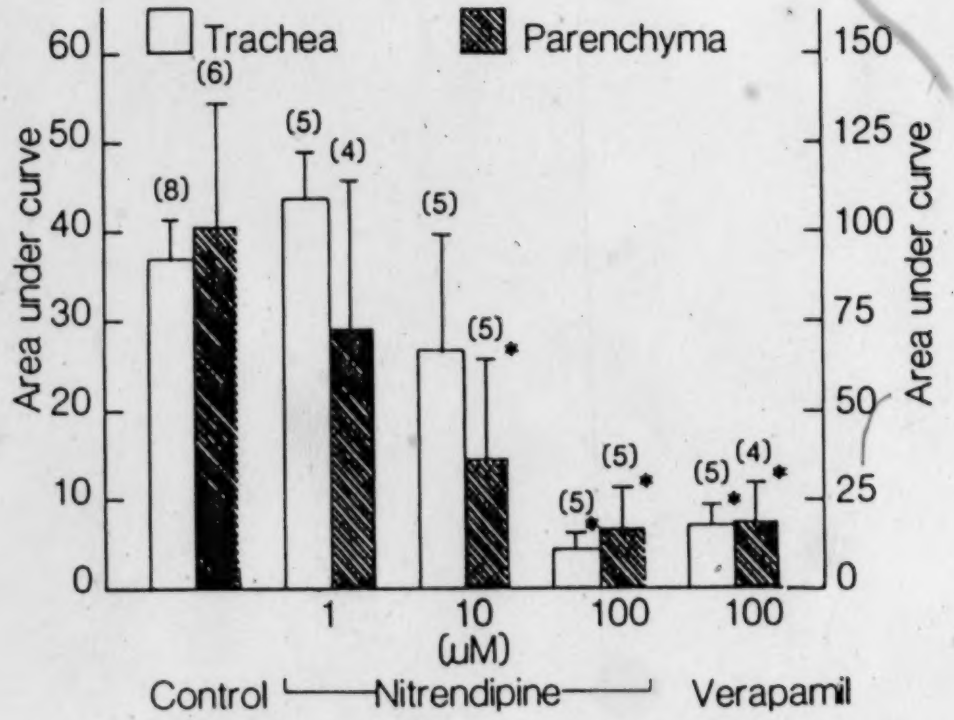


Fig. 31. Effects of nitrendipine and verapamil on AA-induced contractions of trachea (□) and parenchyma (▨). Contractions are expressed as areas under the curve obtained by plotting percentage of maximum histamine against time. *Indicates a significant difference from control ($p<0.05$). Values are mean±SEM of number of experimensts shown above each bar.

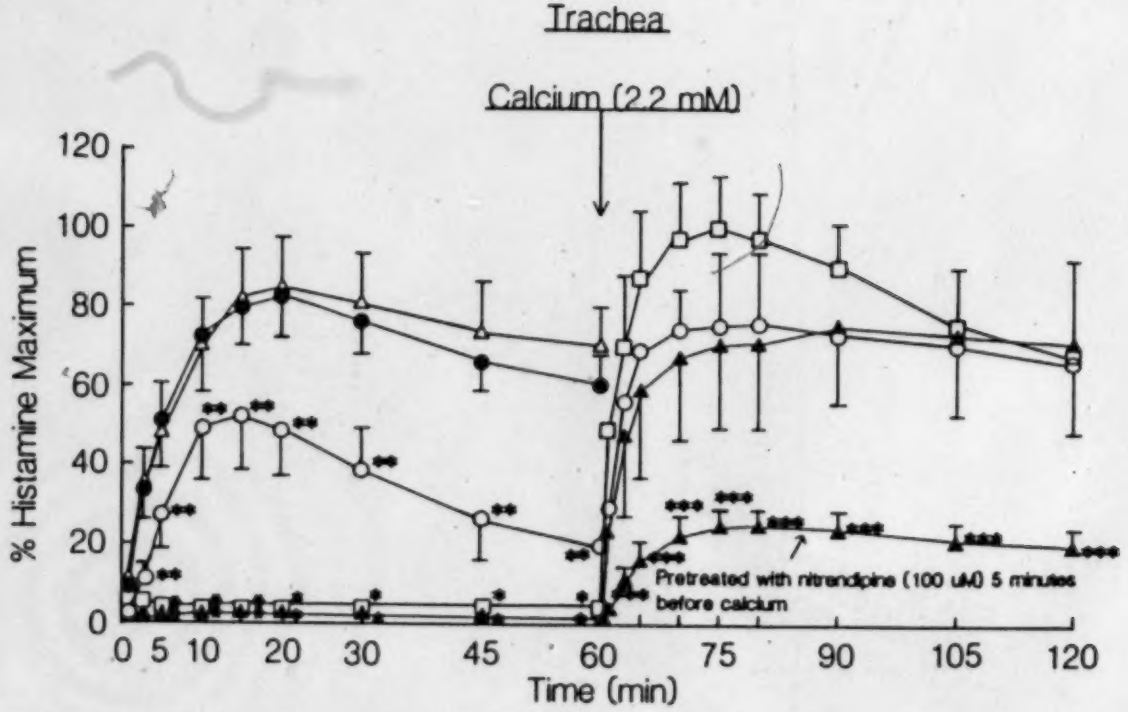


Fig. 32. Effects of lanthanum chloride and EDTA on AA-induced contractions of trachea. Ca^{2+} (2.2 mM) was added at 60 min to the appropriate tissues. The symbols indicate the following: (●) Ca^{2+} (2.2 mM); (○) Ca^{2+}-free; (▲) Ca^{2+}-free plus lanthanum chloride (1 mM); (□) Ca^{2+}-free plus EDTA (300 μM); and (△) Ca^{2+} (2.2 mM) plus lanthanum chloride (1 mM). *Indicates a significant difference from contractions obtained in Ca^{2+}-free KHS ($p < 0.05$). **Indicates a significant difference from control contractions and ($p<0.05$) and ***indicates a significant difference from contractions obtained after calcium was added to lanthanum treated tissues in Ca^{2+}-free KHS ($p<0.05$). The results are the mean±SEM (n=5).

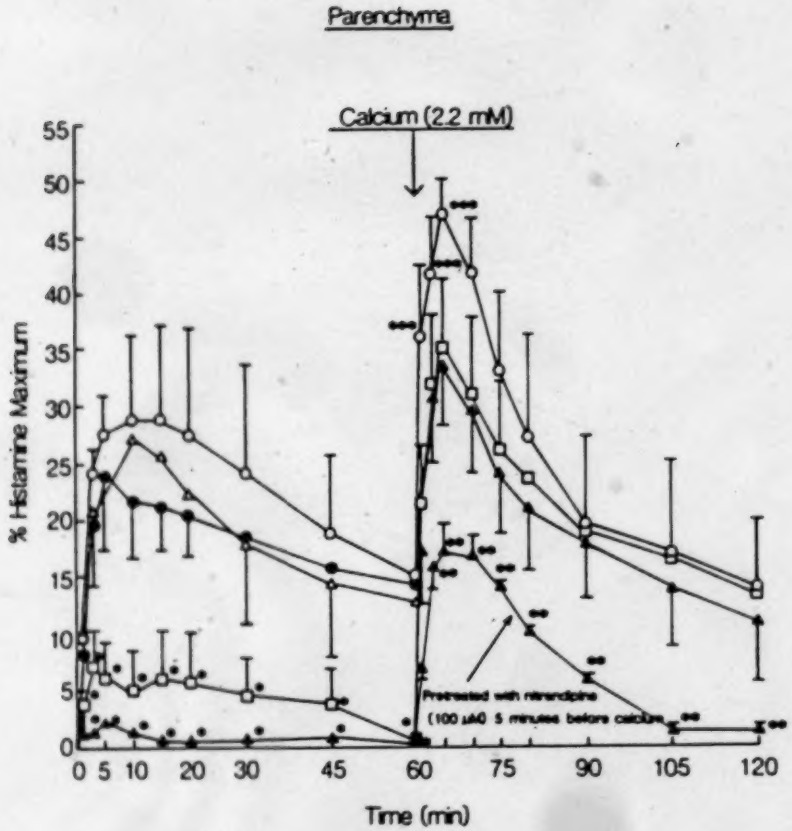


Fig. 33. Effects of lanthanum chloride and EDTA on AA-induced contractions of parenchyma. Ca^{2+} (2.2 mM) was added at 60 min to the appropriate tissues. The symbols indicate the following: (●) Ca^{2+} (2.2 mM); (○) Ca^{2+}-free; (▲) Ca^{2+}-free plus lanthanum chloride (1 mM); (□) Ca^{2+}-free plus EDTA (300 μM); and (△) Ca^{2+} (2.2 mM) plus lanthanum chloride (1 mM). *Indicates a significant difference from contractions obtained in Ca^{2+}-free KHS ($p<0.05$), **indicates a significant difference from contractions obtained after calcium was added to lanthanum-treated tissues in Ca^{2+}-free KHS ($p<0.05$), and ***indicates a significant difference from control contractions. The results are the mean±SEM (n=5).

parenchyma, addition of Ca^{2+} (2.2 mM) to tissues in Ca^{2+}-free KHS caused a statistically significant greater response at early time points after Ca^{2+} addition than that observed with control tissues (Fig. 33). Pretreatment of trachea and parenchyma with nitrendipine (100 μM) 5 min before Ca^{2+} re-addition significantly attentuated the contractile response to this cation (Figs. 32 and 33). In these experiments only tissues stimulated with AA in the presence of lanthanum chloride (1 mM) and Ca^{2+}-free KHS were used. Addition of Ca^{2+} (2.2 mM) to tissues stimulated with AA in normal KHS caused little or no further response of these tissues. Furthermore, addition of more Ca^{2+} to tissues contracting in Ca^{2+}-free KHS after the initial 2.2 mM stimulus at 60 min did not cause any further response (data not shown)

4.3.4 Concentration-response curves to LTC_4:

The concentration-response curves to LTC_4 on the trachea and parenchyma are shown in Fig. 34. Nitrendipine (100 μM) did not significantly affect the response of the trachea to LTC_4 when compared to control. Similar results were obtained with the parenchymal strip. The responses of the trachea to LTC_4 in Ca^{2+}-free KHS and in Ca^{2+}-free KHS with lanthanum (1 mM) were significantly reduced in magnitude ($p<0.05$) when compared to control. However, on parenchyma, only the responses to LTC_4 in Ca^{2+}-free KHS and in the presence of lanthanum chloride (1 mM) were significantly reduced ($p<0.05$; Fig. 34). Addition of calcium (2.2 mM) after the responses to the highest concentra-

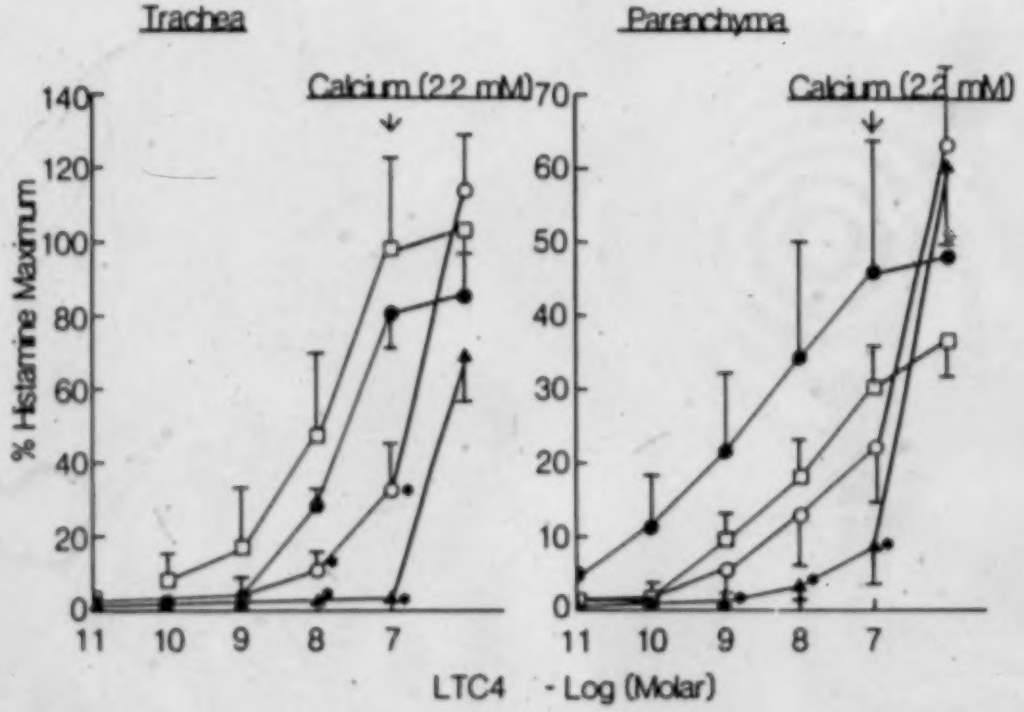


Fig. 34. Contraction of trachea and parenchyma to LTC_4. The symbols indicate the following: (●) Ca^{2+} (2.2 mM); (○) Ca^{2+}-free; (▲) Ca^{2+}-free plus lanthanum chloride (1 mM); and (□) Ca^{2+} (2.2 mM) plus nitrendipine (100 μM). Ca^{2+} (2.2 mM) was added when the response to LTC_4 (0.1 μM) has reached a maximum. *Indicates a significant difference from control responses ($p<0.05$). Results are mean±SEM (n=5).

tion of LTC_4 used (0.1 μM) had plateaued, resulted in an additional contraction of both trachea and parenchyma in Ca^{2+}-free KHS to the control level. Further Ca^{2+} addition did not give further increases in response (data not shown).

4.4 EFFECTS OF LTD_4 ON LUNG AND CEREBELLAR ADENYLATE CYCLASE ACTIVITY

The results presented in this section were calculated as mean±SEM. The SEM between duplicate or triplicate determinations did not exceed 5-10% of the mean value. Hence, in some of the figures the SEM bars have not been displayed in order to enhance clarity. Most experiments have been done at least three times utilizing different membrane preparations.

4.4.1 Initial studies:

The conversion of [^{32}P]-ATP into [^{32}P]-cyclic AMP by guinea-pig lung adenylate cyclase was linear with time (Fig. 35) and protein concentration (up to 1 mg/ml) (Fig. 36). Lung enzyme activity was increased by GTP, isoproterenol, and forskolin (Fig. 37). Forskolin increased enzyme activity by a far greater degree than either GTP or isoproterenol. The effects of GTP were additive to those of forskolin and isoproterenol (Fig. 37).

Figs. 38 and 39 show the concentration-dependent stimulation of lung adenylate cyclase induced by forskolin and isoproterenol. The effects of forskolin were Mg^{2+}-dependent (Fig. 38) since at Mg^{2+} con-

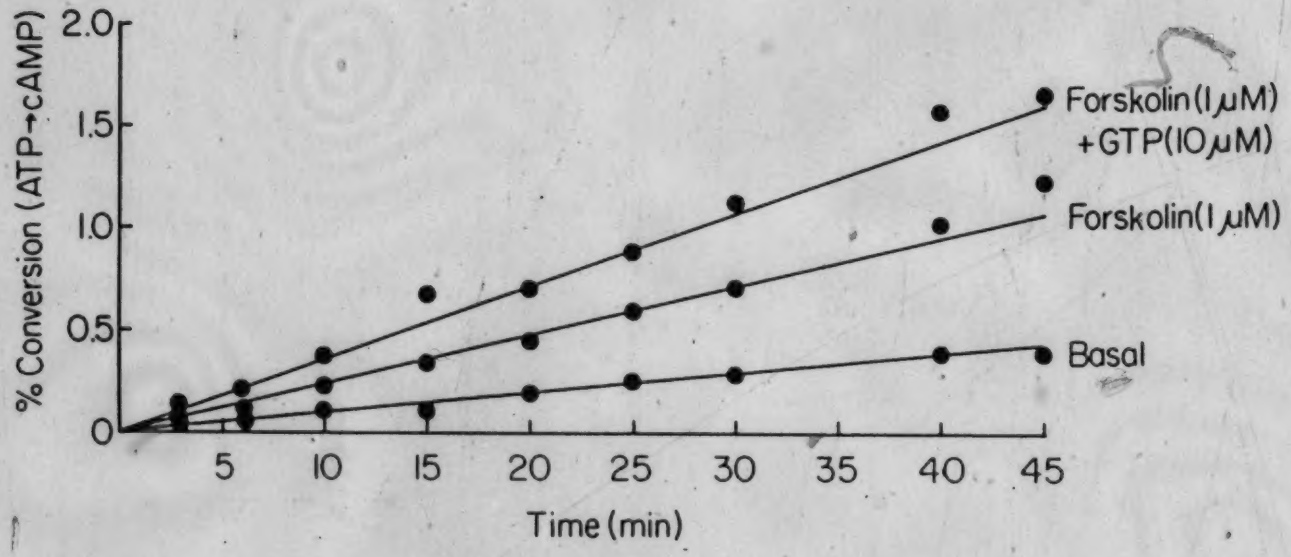


Fig. 35. Time course of activation of lung adenylate cyclase at 20°C. Activation of the enzyme by forskolin and GTP are shown. Assay points are means of duplicates.

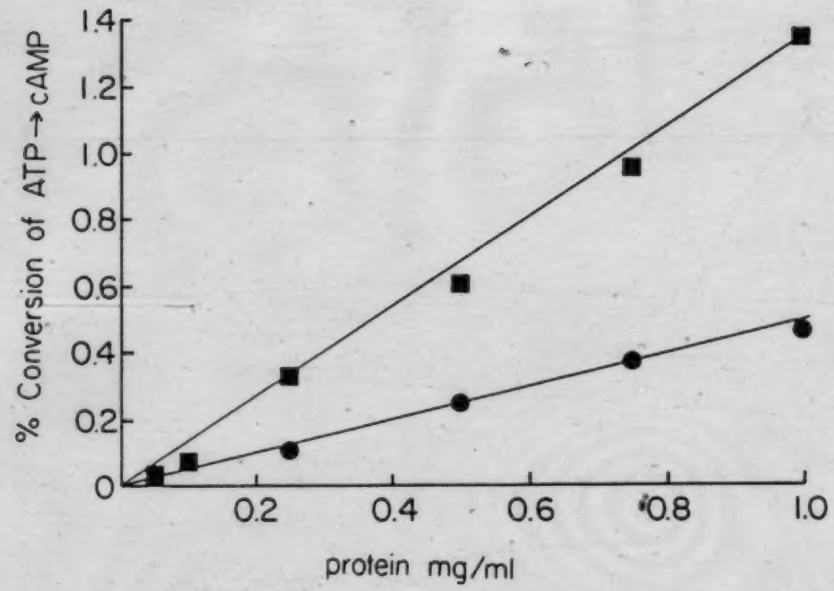


Fig. 36. Protein linearity of adenylate cyclase assays. The percent conversion of [^{32}P]-ATP into [^{32}P]-cyclic AMP at 20°C is shown as a function of added lung protein in the assay. The symbols represent basal activity (●); and forskolin (1 μM) (■) stimulated activity. Values are the means of duplicates.

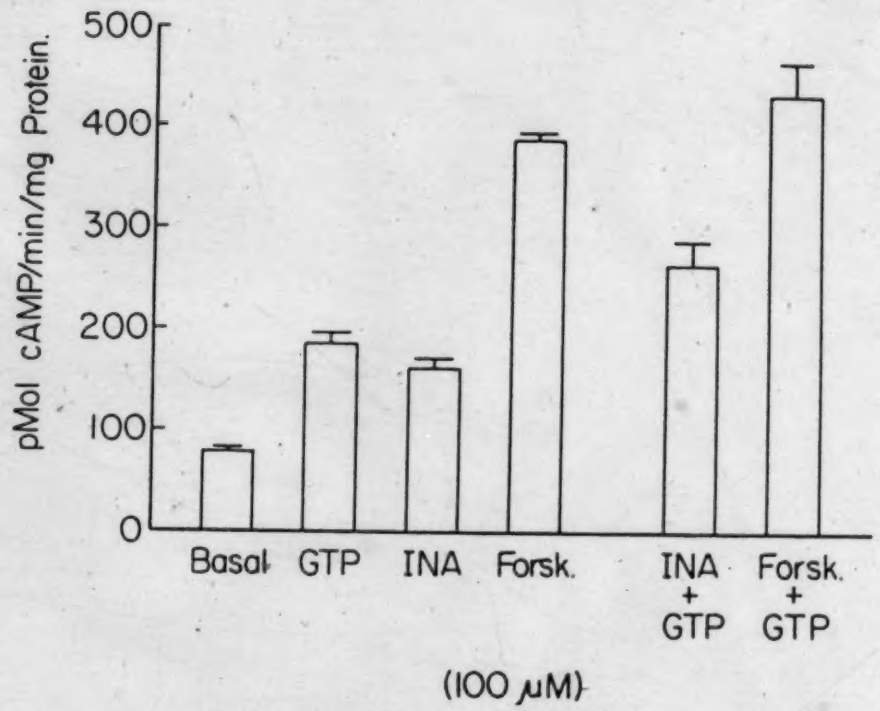


Fig. 37. Activation of lung adenylate cyclase at 37°C by GTP, isoproterenol (INA), and forskolin (Forsk) alone or in combination as shown. Results are mean±SEM of duplicates.

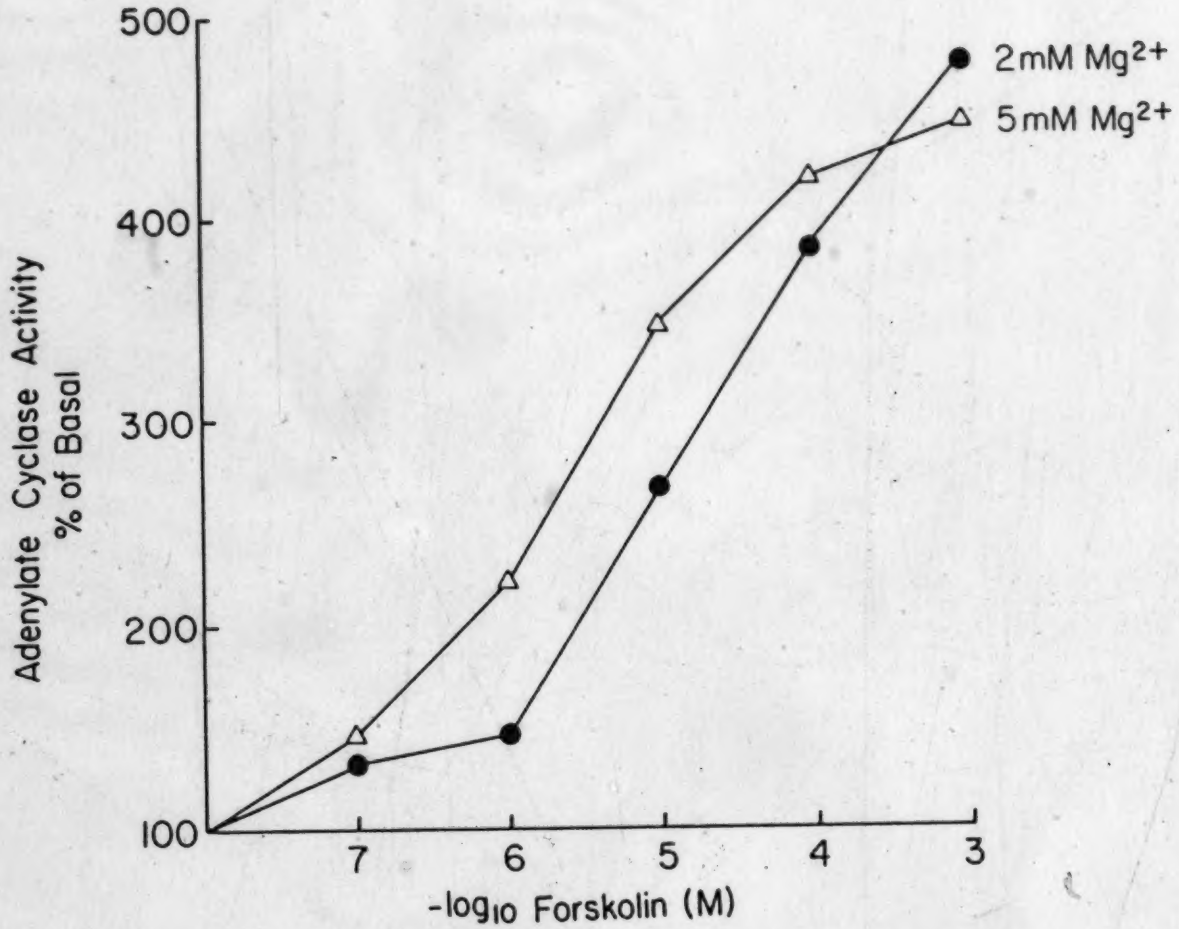


Fig. 38. Stimulation of lung adenylate cyclase by forskolin at 37°C in the presence of Mg^{2+} (2 mM) or (5 mM). The results are the means of duplicates.

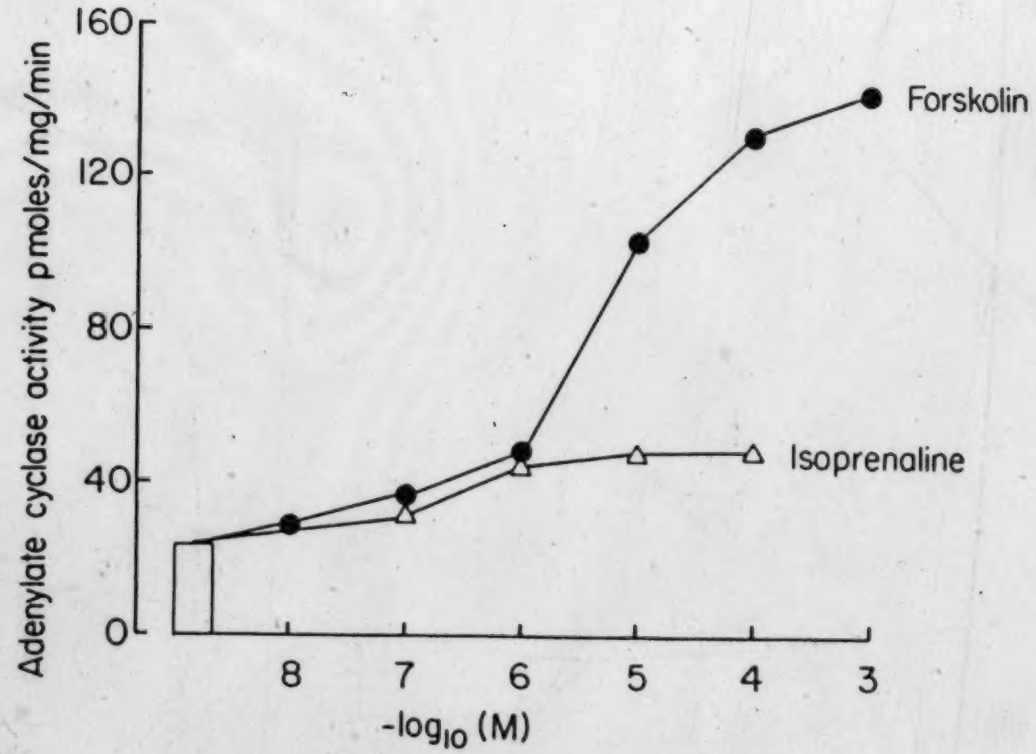


Fig. 39. Stimulation of lung adenylate cyclase at 20°C by forskolin and isoproterenol. The Mg^{2+} concentration was 2 mM. The bar represents the basal unstimulated enzyme activity. Results are the means of duplicates.

centrations of 2 mM the effects of forskolin (expressed as a percentage of basal activity) were less than at Mg^{2+} concentrations of 5 mM. Fig. 39 shows the relatively weak stimulation of the enzyme induced by isoproterenol at 20°C and the dramatic stimulation induced by forskolin. The EC_{50} of forskolin in stimulating the lung enzyme was approximately 10 μM (Figs. 38 and 39).

The activation of lung adenylate cyclase by GTP in the absence and presence of LTD_4 (0.1 μM) is shown in Fig. 40. Table 16 shows the effects of adenosine deaminase (10 U/ml) on the GTP-stimulated enzyme. Adenosine deaminase was included in order to determine whether adenosine was being formed endogenously in the assay. This is a common problem in studies of adenylate cyclase because endogenous adenosine may mask stimulation or inhibition of the enzyme by other agents. Adenosine deaminase will deaminate adenosine to produce inosine which is virtually inactive at adenosine receptors. As can be seen from Table 16 inclusion of adenosine deaminase did not significantly alter the profile of GTP stimulation of the enzyme.

Inclusion of adenosine deaminase in rat cerebellar cyclase assays completely changed the profile of the effects of GTP (Figs. 41 and 42). It can be seen in Fig. 41 that the higher the amount of enzyme (protein concentration) added, the greater was the inhibitory effect of GTP at any given concentration. However, if adenosine deaminase (2.5 or 5 U/ml) was also included, the inhibitory effects of GTP were reduced (Fig. 41). Hence it seemed that endogenously formed adenosine

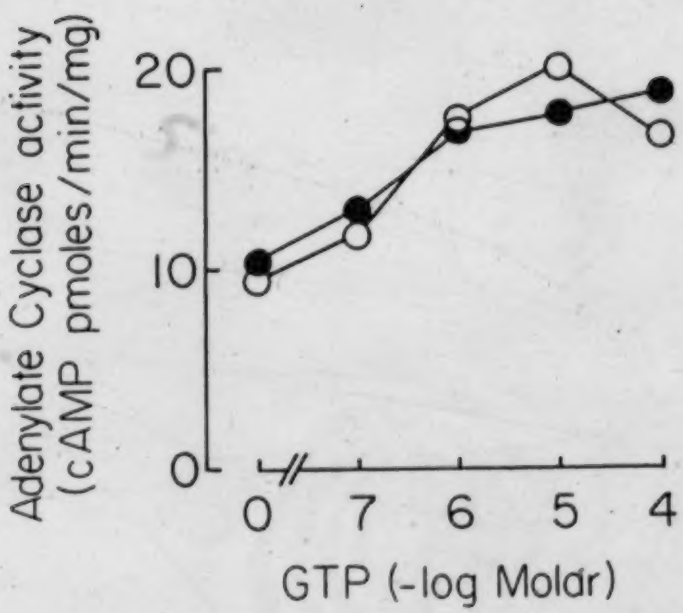


Fig. 40. Activation of lung adenylate cyclase by GTP in the absence (●) and presence of LTD_4 (0.1 μM) (○) at 20°C. The points are means of duplicates.

Table 16. Effects of GTP on lung adenylate cyclase in the presence or absence of Adenosine deaminase (10 U/ml).

	Control	+ Adenosine deaminase (10 U/ml)
	Pmoles cyclic AMP/min/mg protein	
Basal activity	11.5±1.2[a]	10.4±1.2
GTP 0.1 (μM)	14.2±0.6	12.6±0.1
GTP 1.0 (μM)	17.3±0.1	16.7±1.5
GTP 10.0 (μM)	21.1±0.7	17.2±0.7
GTP 100.0 (μM)	20.5±0.4	18.5±0.9

[a] = values are mean±SEM of duplicates.

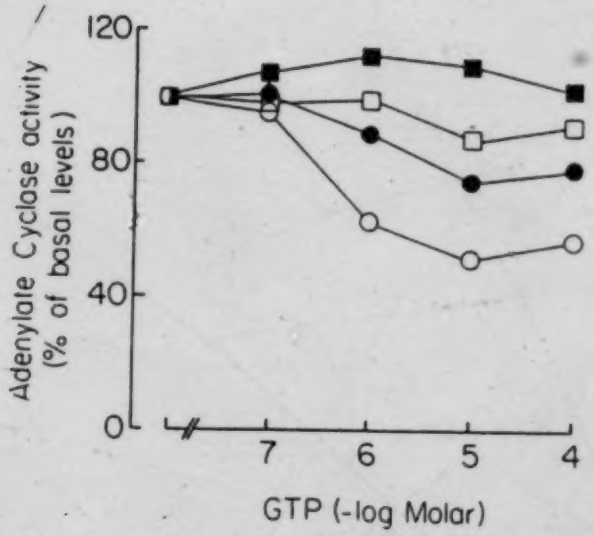


Fig. 41. The effects of adenosine deaminase on the responses of rat cerebellar adenylate cyclase to GTP. The symbols represent the following: (○), 0.4 mg/ml protein; (●), 0.2 mg/ml protein; (□), 0.4 mg/ml protein plus adenosine deaminase 2.5 U/ml; (■), 0.4 mg/ml protein plus adenosine deaminase 5 U/ml. The experiment was performed at 20°C. Each point is the mean of duplicates.

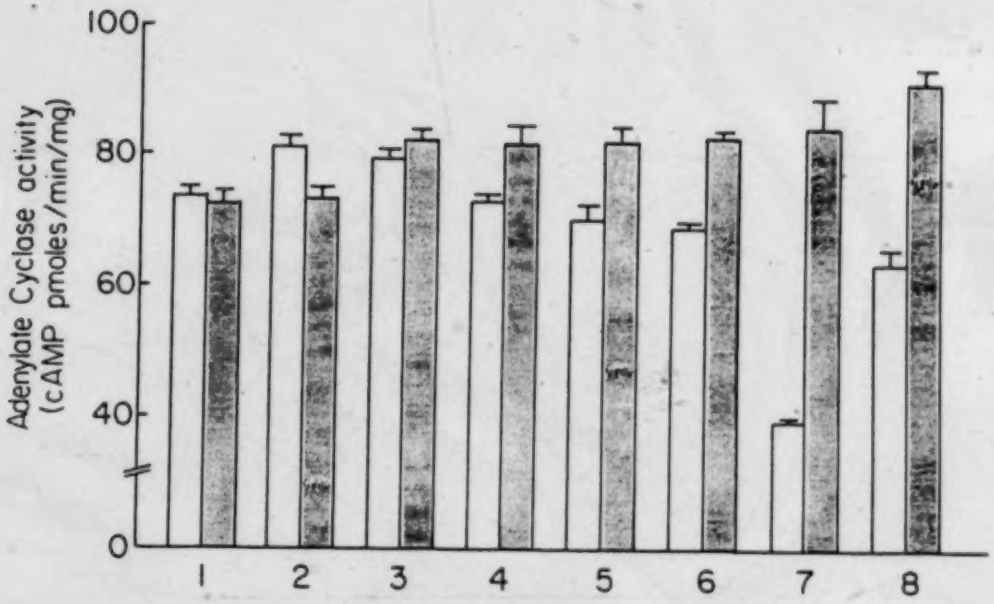


Fig. 42. Effects of adenosine deaminase on the responses of rat cerebellar adenylate cyclase to GTP and adenosine (10 μM). The clear bars are controls. The hatched bars are in the presence of adenosine deaminase (5 U/ml). The numbers indicate the following: 1: Basal activity; 2: adenosine; 3: GTP (0.1 μM); 4: GTP (0.1 μM) + adenosine; 5: GTP (1 μM); 6: GTP (10 μM); 7: GTP (10 μM) + adenosine; 8: GTP (100 μM). The results are mean±SEM of duplicates.

was inhibiting the enzyme in a GTP-dependent fashion. This was confirmed by the experiment shown in Fig. 42. Exogenously added adenosine inhibited the enzyme (Bars 6 and 7) in the presence of GTP and absence of adenosine deaminase. However, if adenosine deaminase (5 U/ml) was present, the inhibitory effects of adenosine were abolished and the slight stimulatory effects of GTP on rat cerebellar adenylate cyclase became apparent (Figs. 41 and 42). Therefore, in all further experiments with cerebellar adenylate cyclase only low amounts (<0.2 mg/ml) of protein were used. Furthermore, adenosine deaminase (5 U/ml) was present in the assay mixture. It is apparent that endogenously produced adenosine would inhibit the enzyme and mask the effects of other inhibitors.

Table 17 shows the basal (unstimulated) adenylate cyclase activities of lung and cerebellum. The influence of Mg^{2+}, Mn^{2+}, temperature and substrate concentration are shown. As shown cerebellar cyclase specific activity was about 8 fold higher than for lung cyclase under similar conditions (Table 17). Furthermore, the specific activity of both enzymes was stimulated by an increase in temperature and divalent cation concentration. Mn^{2+} was more effective than Mg^{2+} in stimulating specific cyclase activity. Finally, a reduction of substrate concentration (from 0.1 mM to 0.05 mM) only slightly reduced the specific activity of cerebellar cyclase. This was done in order to further reduce the formation of endogenous adenosine in cerebellar cyclase assays.

Table 17. Basal Adenylate cyclase activities (cyclic AMP pmoles/min/mg)

Tissue	Cation (mM)	Temp (°C)	ATP (mM)	Adenylate cyclase activity
Guinea pig	Mg^{2+} 2.0	20.0	0.1	14.2±1.1 (16)[a]
lung	Mg^{2+} 2.0	37.0	0.1	33.1±5.6 (6)
	Mg^{2+} 5.0	37.0	0.1	64.8±3.6 (12)
	Mn^{2+} 1.0	20.0	0.1	31.9±1.7 (5)
Rat cerebellum	Mg^{2+} 2.0	20.0	0.1	107.1± 7.3 (12)
(+AD 5 U/ml)	Mg^{2+} 2.0	20.0	0.05	95.6± 7.9 (5)
	Mg^{2+} 5.0	20.0	0.05	138.2±11.0 (5)
	Mg^{2+} 1.0	20.0	0.05	230.0±13.3 (3)

[a] = mean±SEM (number of preparations).

4.4.2 Effects of LTD_4 on lung adenylate cyclase:

A major problem in these studies was a difficulty in obtaining reproducible data. This difficulty was compounded by the fact that the LTD_4 effects on the enzyme were small. In experiments performed during this work, LTD_4 effects varied from 0 to 60 % inhibition with the latter occurring very rarely. However, inhibitory effects of about 10-15 % occurred regularly. Representative results are included of all these situations.

The lack of effect of LTD_4 (0.1 μM) on the forskolin and isoproterenol-stimulated lung enzyme is shown in Fig. 43. As can be seen LTD_4 did not affect the degree of stimulation by either forskolin or isoproterenol. In the presence of GTP (10 μM) and forskolin (10 μM) (Fig. 44) LTD_4 slightly inhibited the enzyme with a maximal effect occurring at about 0.1 μM. As can be seen from the figure there was a variable effect of LTD_4 between three different preparations of the enzyme. Furthermore, higher concentrations of LTD_4 (1 μM) tended to inhibit less than lower concentrations.

The effects of LTD_4 on the forskolin (10 μM) stimulated enzyme in the presence of GTP (10 μM) or GTP (10 μM) and NaCl (100 mM) are shown in Fig. 45. As shown, LTD_4 inhibited the enzyme with somewhat greater effects in the presence of NaCl. NaCl also reduced enzyme activity on its own. This effect of LTD_4 shown in Fig. 45 was difficult to reproduce in the same preparation or in other preparations. Similarly the experiment shown in Fig. 46 was difficult to

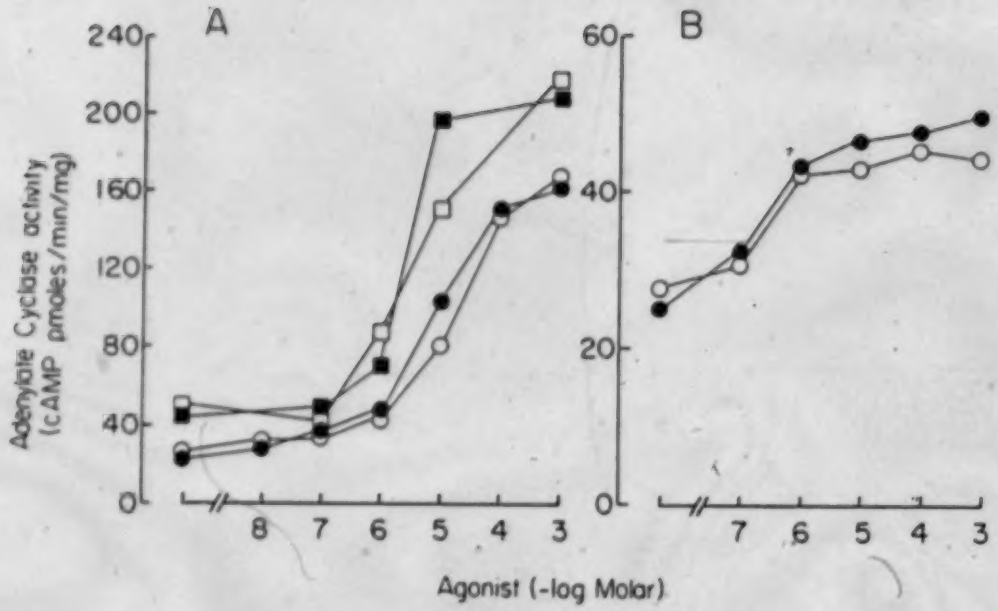


Fig. 43. Lack of effect of LTD_4 (0.1 μM) on forskolin (panel A) or isoproterenol stimulated lung adenylate cyclase (panel B) at 20°C. Forskolin stimulation was measured at Mg^{2+} (2 mM) (●) or Mg^{2+} (5 mM) (■). Isoproterenol stimulation was measured at Mg^{2+} (2 mM). The shaded symbols were the controls and the open symbols were in the presence of LTD_4 (0.1 μM). Each point is the mean of duplicates.

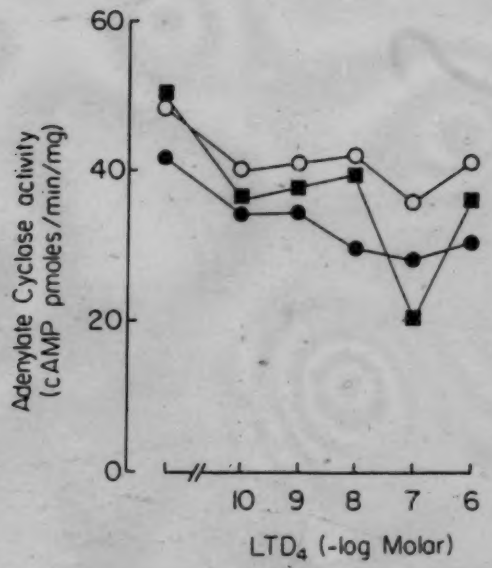


Fig. 44. Effect of LTD_4 on forskolin-stimulated lung adenylate cyclase. GTP (10 μM) was also present. The three different symbols represent three different preparations. Each point is the mean of duplicates.

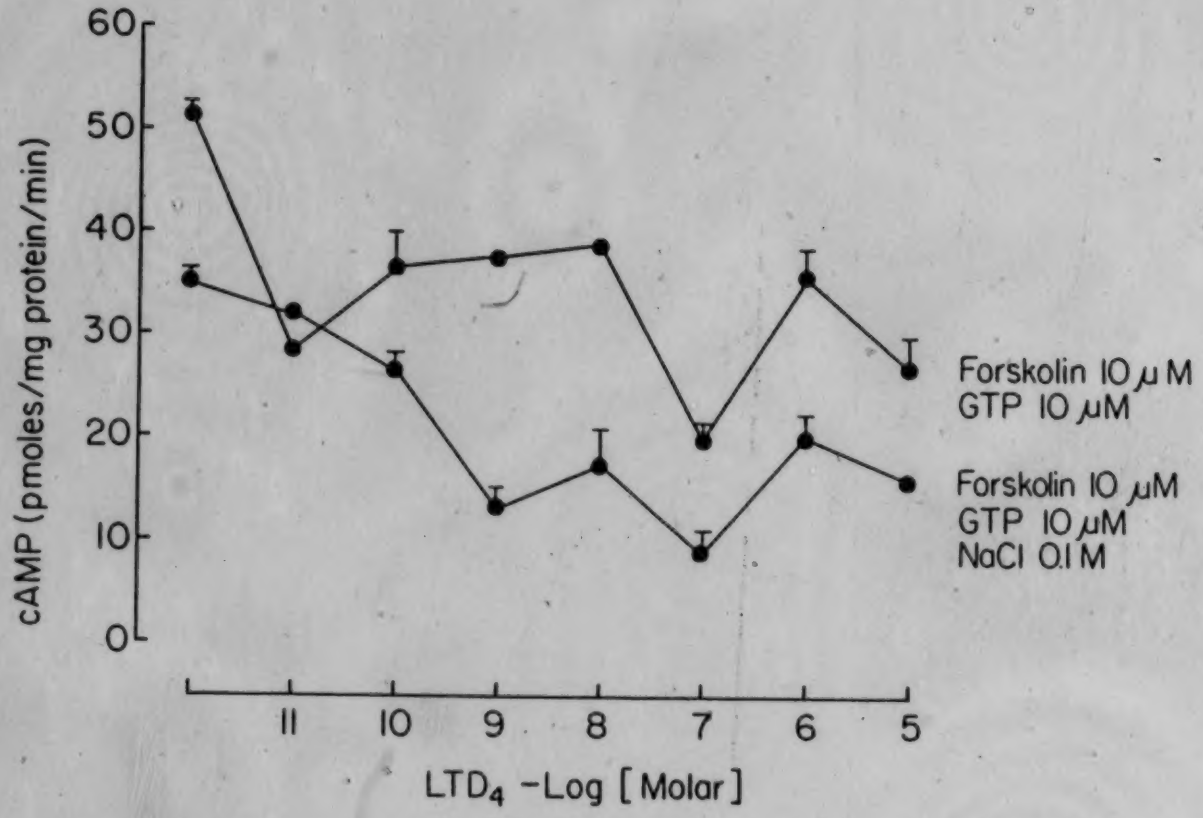


Fig. 45. Effect of LTD_4 on lung adenylate cyclase at 20°C in the presence of Mg^{2+} (2 mM). Each point is the mean±SEM of triplicates. See text for details.

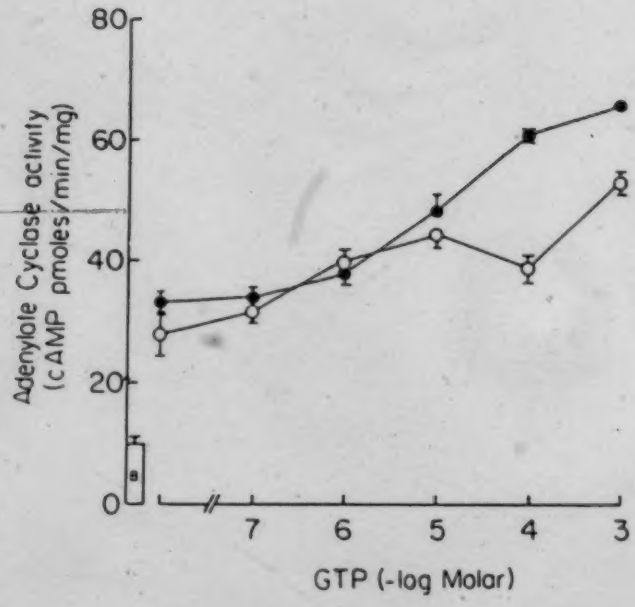


Fig. 46. Effect of GTP on LTD_4-induced inhibition of lung adenylate cyclase. Forskolin (1 μM) was present in each tube. The symbols indicate control (●) and LTD_4 (0.1 μM) (○). The points are mean±SEM of duplicates. The assay was at 20°C in the presence of Mg^{2+} (2 mM). The enzyme's basal activity is indicated by the bar.

reproduce. This experiment was done to demonstrate the GTP dependence of LTD_4 (0.1 μM) inhibition of lung adenylate cyclase.

The effects of LTD_4 on the forskolin (10 μM)-stimulated enzyme in the presence of GTP 0.1, 1.0 and 10 (μM) are shown in Fig. 47. This experiment was performed at 37°C in the presence of Mg^{2+} (5 mM). As shown, LTD_4 inhibited the enzyme with no apparent concentration dependency. However, the magnitude of inhibition was greater at the higher GTP concentrations (1 and 10 μM). Yet again, these results were difficult to reproduce.

The effect of LTD_4 on the lung enzyme at 20°C in the presence of Mn^{2+} (1 mM) and GTP (0.1 μM and 10 μM) are shown in Fig. 48. Also shown is the effect of LTD_4 on the basal activity in the presence of Mg^{2+} (2 mM). Small inhibitory and stimulatory effects of LTD_4 could be demonstrated. Mn^{2+} (1 mM) increased the basal activity of the enzyme to 3 fold the level observed with Mg^{2+} (Fig. 48). In the presence of GTP (10 μM); LTD_4 (10 nM) slightly inhibited the enzyme. It is noteworthy that the same concentration of LTD_4 slightly stimulated the enzyme in the absence or presence of low concentrations of GTP (0.1 μM) or inhibited the enzyme slightly in the presence of Mg^{2+} (Fig. 48).

In summary, LTD_4-induced inhibition of lung adenylate cyclase was small and difficult to reproduce. However, an effect occurred in most of the experiments performed even though this effect was sometimes small. This is illustrated in Fig. 49 which should be

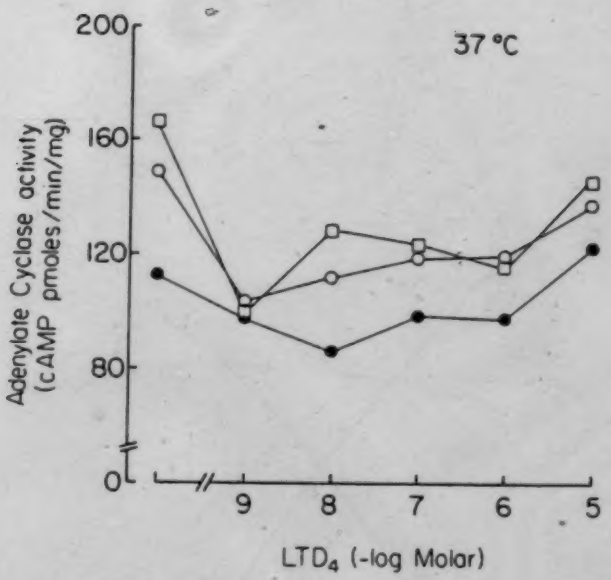


Fig. 47. Effect of LTD_4 on lung adenylate cyclase activity. Forskolin (10 μM) was present in each tube. The symbols indicate the presence of GTP (0.1 μM) (●), (1.0 μM) (○), and (10 μM) (□). The assay was conducted at 37°C in the presence of Mg^{2+} (5 mM). Each point is the mean of duplicates.

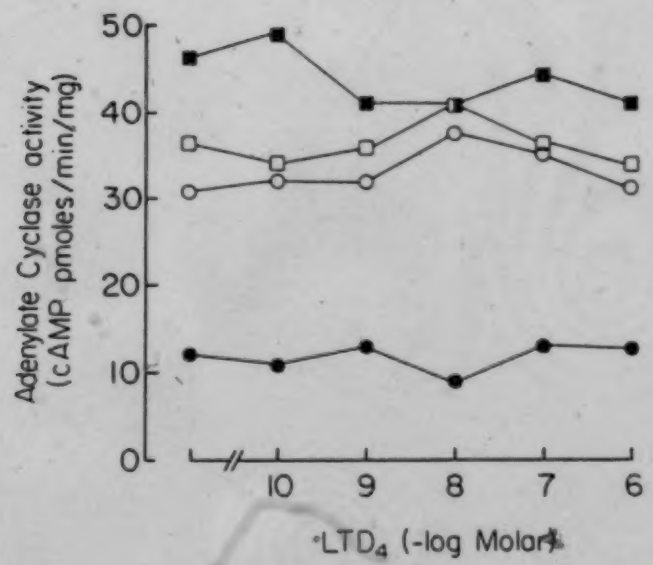


Fig. 48. Effect of LTD_4 on lung adenylate cyclase activity in the presence of Mg^{2+} (2 mM) or Mn^{2+} (1 mM). The symbols indicate Mg^{2+} (●), Mn^{2+} (○), Mn^{2+} and GTP (0.1 μM) (□), and Mn^{2+} and GTP (10 μM) (■). The assay was at 20°C. Each point is the mean of duplicates.

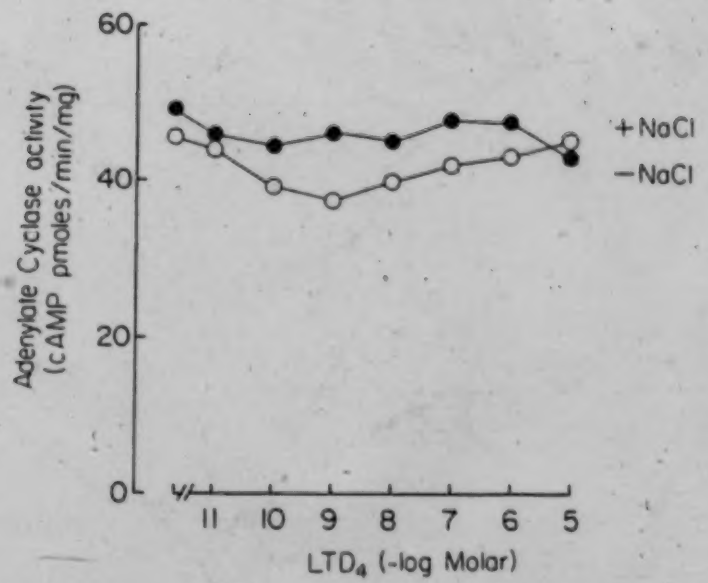


Fig. 49. Effect of LTD_4 on lung adenylate cyclase at 20°C in the presence of Mg^{2+} (2 mM). Each tube contained 10 μM each of forskolin and GTP and NaCl 100 mM as indicated in the figure. Each point is the mean of duplicates.

compared with the experiment in Fig. 45. Both experiments were done under identical conditions as indicated in the figure legends.

In order to determine the causes of variation and lack of reproducibility of the data presented above, a variety of experimental approaches to membrane preparation were undertaken. These included very gentle homogenization procedures which utilized hand homogenization of the tissue or homogenization at low motor speeds. Furthermore, the pH of the homogenization medium was varied from pH 5.5 to pH 8.5. None of these approaches yielded greater inhibition by LTD_4.

Another approach taken was to test the hypothesis that the LTD_4 may not be binding to its receptors in the membranes used for assay of adenylate cyclase activity. Hence, this would lead to little inhibitory effects on enzyme activity. Therefore, the specific binding of $[^3H]$-LTC_4 to guinea-pig lung membranes was studied. The method involved incubation of $[^3H]$-LTC_4 with lung membranes under conditions exactly similar to adenylate cyclase assay conditions. $[^3H]$-LTC_4 bound specifically and with high affinity to lung membranes (Fig. 50). The B_{max} and k_D value demonstrated for the binding interaction are similar to published values (Pong & DeHaven, 1984).

Finally, since divalent cations (mM concentrations) have been shown to stimulate binding of LTs to their receptors, the effects of Ca^{2+} on adenylate cyclase activity of guinea-pig lung (Fig. 51) and rat cerebellum (Fig. 52) were investigated. The effects of Ca^{2+} on

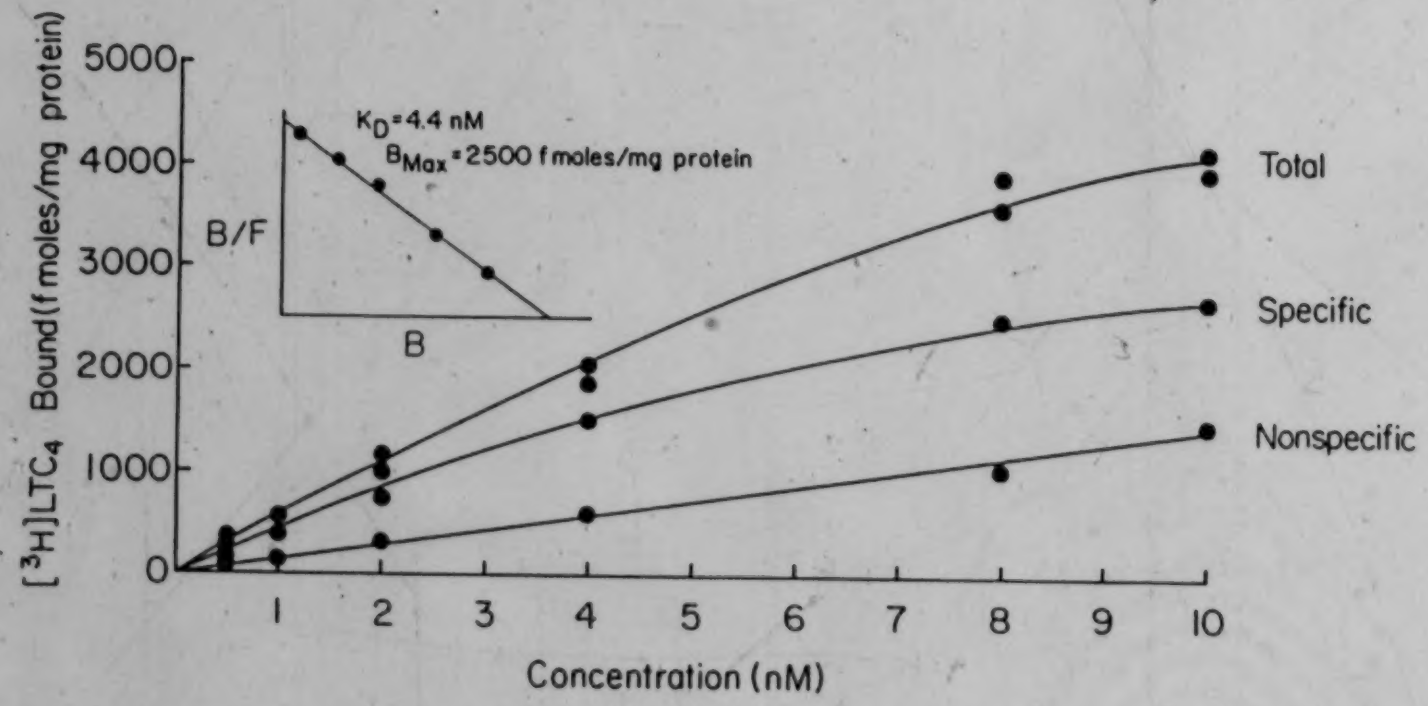


Fig. 50. Binding of [^{3}H]-LTC$_4$ to guinea pig lung membranes at 20°C. The points are means of duplicate values. The inset shows the Scatchard representation of the data.

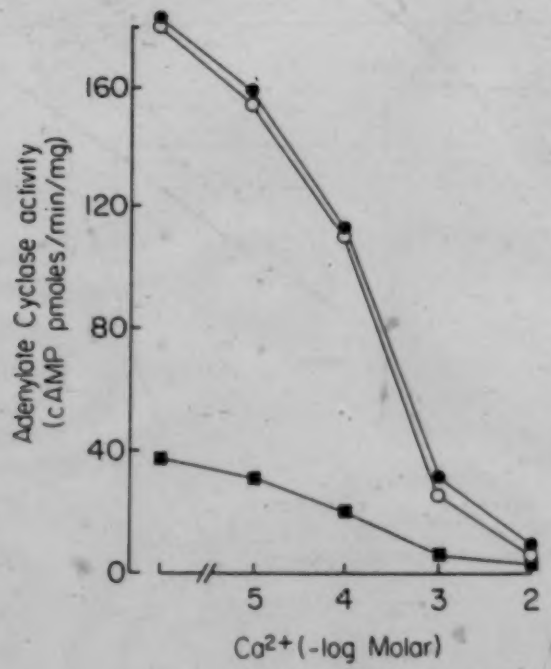


Fig. 51. Inhibition lung adenylate cyclase activity by Ca^{2+}. The symbols represent basal activity (■); forskolin and GTP (10 μM) (●), and forskolin and GTP (10 μM each) with LTD_4 (0.1 μM) (○). The points are the means of duplicates. The assay was carried out at 37°C.

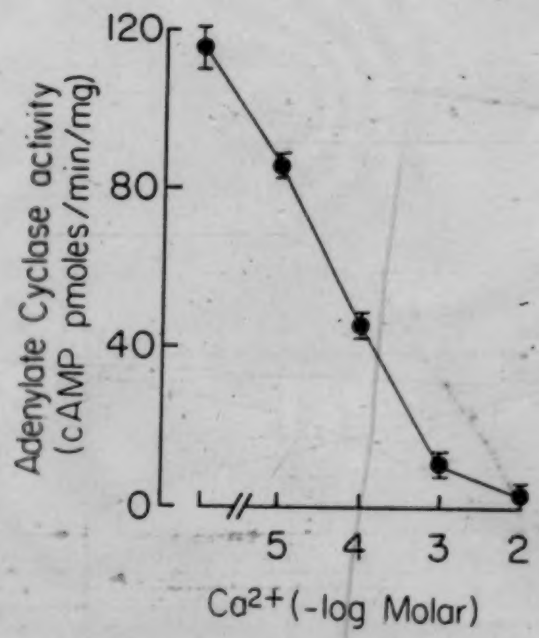


Fig. 52. Inhibition of cerebellar basal adenylate cyclase activity by Ca^{2+}. The points are mean±SEM of duplicates. The assay was carried out at 20°C in the presence of Mg^{2+} (2 mM).

LTD_4 action on lung cyclase was also studied (Fig. 51). Ca^{2+} inhibited basal and forskolin stimulated activity of lung adenylate cyclase and basal activity of cerebellar adenylate cyclase with an IC_{50} of about 100 μM (Figs. 51 and 52). Hence Ca^{2+} ions could not be used to stimulate the binding of LTD_4 to its receptors in order to facilitate the effect on adenylate cyclase.

4.4.3 Effect of GTPγS on guinea-pig lung and rat cerebellar adenylate cyclase:

As mentioned in section 1.2.3.1.1. GTPγS-induced inhibition of adenylate cyclase activity is an index of the presence and function of Ni (Seamon & Daly, 1982; Jakobs et al. 1984). This effect of GTPγS was utilized in these studies to demonstrate Ni functions in both cyclase systems studied. Furthermore, this effect was used to demonstrate optimal conditions for inhibiton of both enzymes.

The effects of GTPγS were determined against the basal and forskolin stimulated activity of lung and cerebellar cyclases at 20°C and 37°C. As shown in Fig. 53, GTPγS slightly inhibited forskolin stimulated but not basal adenylate cyclase activity of lung at 20°C. The magnitude of adenylate cyclase inhibition was 16.3±3.4% (n=3) (Table 18). This maximal inhibitory effect of GTPγS occurred at 1 nM. Higher concentrations stimulated enzyme activity (Fig. 53). The presence of LTD_4 (0.1 μM) did not modify GTPγS effects (Fig. 53).

GTPγS inhibition of forskolin-stimulated lung adenylate cyclase

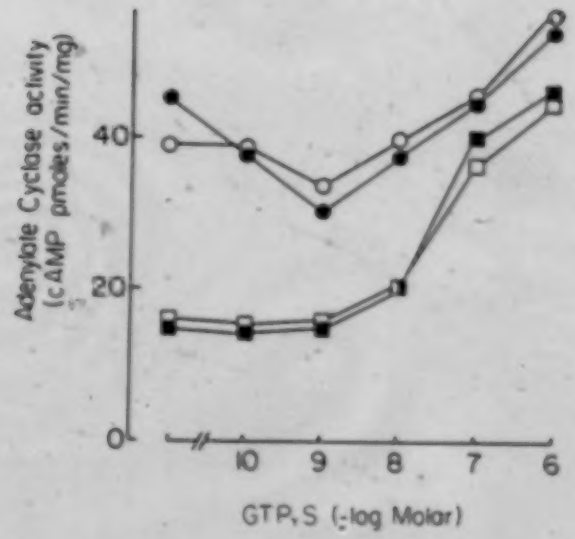


Fig. 53. Inhibition and stimulation of lung adenylate cyclase activity by GTPγS at 20°C. The Mg^{2+} concentration was 2 mM. The squares represent basal unstimulated activity. The circles represent stimulation with forksolin (10 μM). Open symbols represent activity determined in the presence of LTD_4 (0.1 μM) and the shaded symbols, control activity. Each point is the mean of duplicates.

Table 18. GTPγS inhibition[a] of adenylate cyclase activity.

Tissue	Temp (°C)	ATP (mM)	% inhibition of adenylate cyclase activity
Rat cerebellum	20.0	0.1	60.2±3.6 (6)[b]
+			
GTPγS (0.1 μM)	20.0	0.1 + AD[c] 5 U/ml	62.6±4.4 (4)
	20.0	0.05 + AD 5 U/ml	61.3±1.8 (4)
Guinea pig lung	20.0	0.1	16.0±3.4 (3)[d]
+			
GTPγS (1 nM)	37.0	0.1	3.9±1.3 (2)[c]

a = Measured in the presence of Mg^{2+} (2 mM).

b = Mean±SEM (number of preparations).

c = AD = adenosine deaminase.

d = Measured in the presence of forskolin (100 μM).

activity at 37°C was very small 3.9±1.3% (n=2) (Table 18, Fig. 54). Concentrations over 1 nM stimulated the enzyme and LTD_4 (0.1 μM) did not modify this effect (Fig. 54).

Fig. 55 shows the effects of GTPγS on lung and cerebellar adenylate cyclase activities at 37°C. As shown GTPγS inhibited and stimulated enzyme activity in a concentration dependent fashion. Low concentrations (0.1 μM) inhibited cerebellar adenylate cyclase stimulated with forskolin (100 μM) to about 50% of the control level. The maximal inhibition of cerebellar adenylate cyclase in the absence of forskolin stimulation was less (Fig. 55) and occurred at a lower concentration (10 μM) of GTPγS. GTPγS stimulated adenylate cyclase activity of cerebellar membranes at higher concentrations (Fig. 55).

The time course of GTPγS inhibition of basal cerebellar adenylate cyclase activity at 20°C in the presence of low Mg^{2+} (2 mM) concentrations is shown in Fig. 56. The inhibition proceeded after an initial lag phase of 5 minutes. The magnitude of inhibition at the maximally effective concentration (0.1 μM) was 62.6±4.4% (n=4) (Table 17).

The small inhibitory effect of GTPγS on lung adenylate cyclase relative to cerebellar adenylate cyclase (Table 18) implies that Ni function or concentrations are relatively smaller in lung than in cerebellar membranes. It was important to determine whether this situation was intrinsic to lung membranes and is not an artifact of lung membrane preparation. Ni effects are reduced by proteases (Jakobs et al. 1984) and homogenization of lung and other tissues may

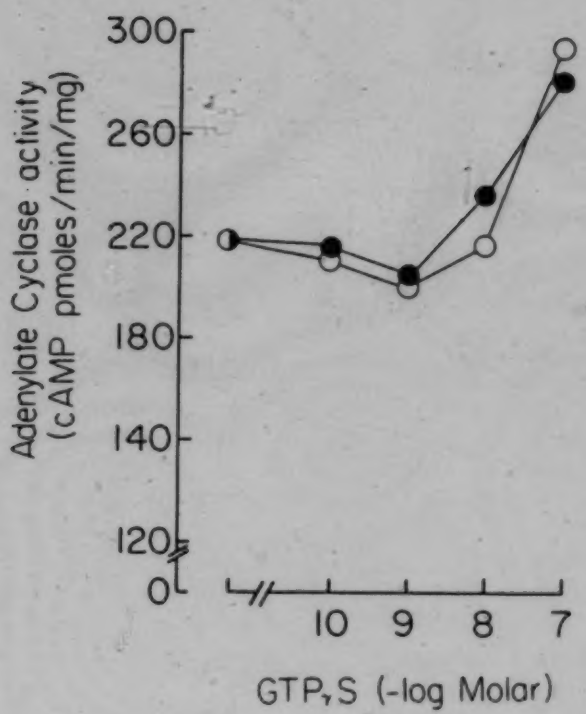


Fig. 54. Effect of GTPγS on forskolin (100 μM) stimulated lung adenylate cyclase activity at 37°C. The Mg^{2+} concentration was 2 mM. Open symbols represent activity in the presence of LTD_4 (0.1 μM). Shaded symbols represent control activity. Each point is the mean of duplicates.

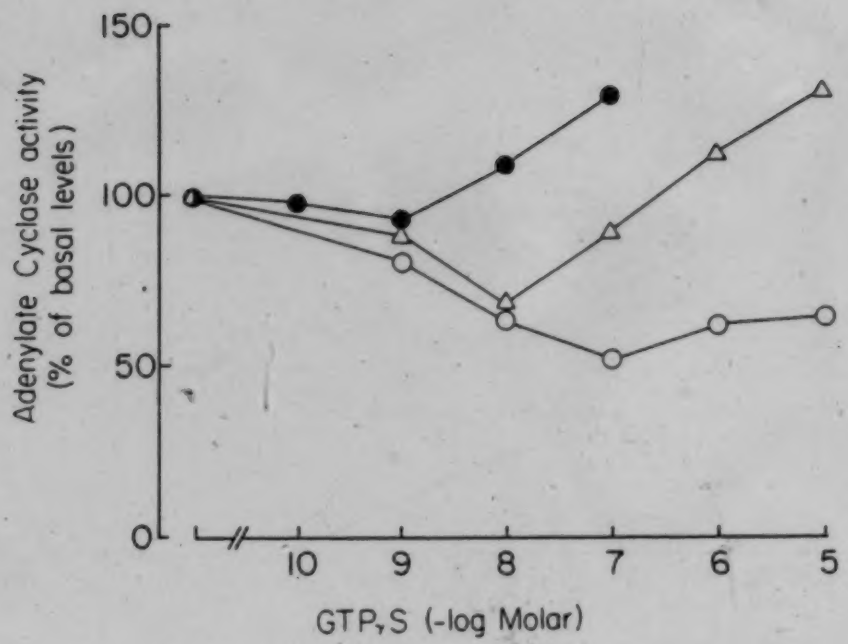


Fig. 55. Effect of GTPγS on lung and cerebellar adenylate cyclase activities at 37°C. The Mg^{2+} concentration was 2 mM. The symbols represent lung adenylate cyclase stimulated with forskolin (●); cerebellar adenylate cyclase at basal activity (△); and cerebellar adenylate cyclase stimulated with forskolin (100 μM) (○). The control adenylate cyclase activities were 218.5±3.0, 147.0±10.1, and 964.5±24.1 pmoles cyclic AMP/min/mg, respectively. Each point is the mean of duplicates.

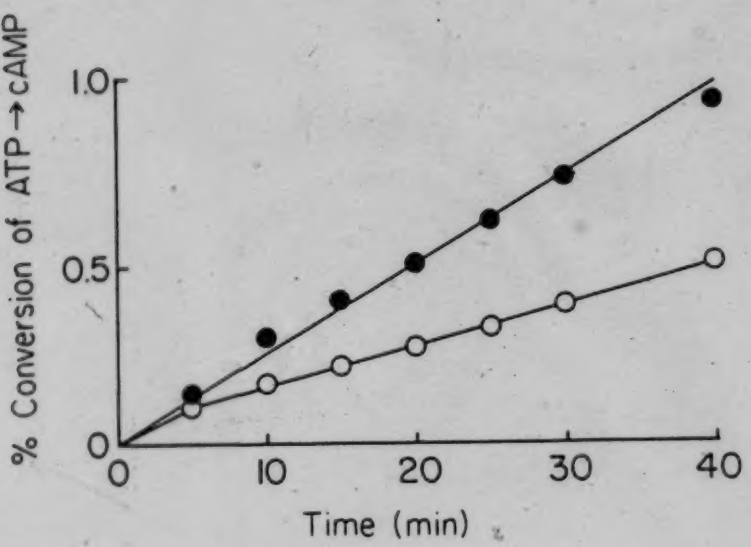


Fig. 56. Time course of GTPγS (0.1 μM) (○) inhibition of basal (●) cerebellar adenylate cyclase activity at 20°C. The Mg^{2+} concentration was 2 mM. Each point is the mean of duplicates.

release proteases that will affect Ni function. Therefore, to show that such a situation was not occurring in these experiments, the supernatant from a lung homogenate was taken and incubated with half a rat cerebellum. The tissue was homogenized in this medium and membranes prepared as described in the Methods. The other half of the cerebellum was treated exactly the same way with buffer that had not been exposed to lung tissue. Subsequently, both membrane preparations were assayed for GTPγS-induced inhibition of adenylate cyclase activity at 20°C. GTPγS inhibited basal adenylate cyclase activities of both preparations with exactly similar percentage inhibition at all concentrations (Fig. 57). The basal activities for both preparations were also very similar (Fig. 57).

Hence using this bioassay approach it is likely that the small Ni-mediated inhibition of lung adenylate cyclase is an intrinsic quality of the membranes and is not due to artefacts arising from lung homogenization . Furthermore, the use of protease inhibitors including polymethylsulphonyl fluoride (0.3 mM), soybean trypsin inhibitor (20 μg/ml), leupeptin (1 μg/ml) and bacitracin (100 μM) in the homogenization buffer did not increase the magnitude of GTPγS inhibition of lung adenylate cyclase activity (Fig. 58). However, basal activities and forskolin stimulated activities were slightly increased after homogenization in the presence of protease inhibitors (Fig. 58).

Therefore, from the above experiments it seems likely that the small degree of LTD_4-induced inhibition of lung adenylate cyclase may

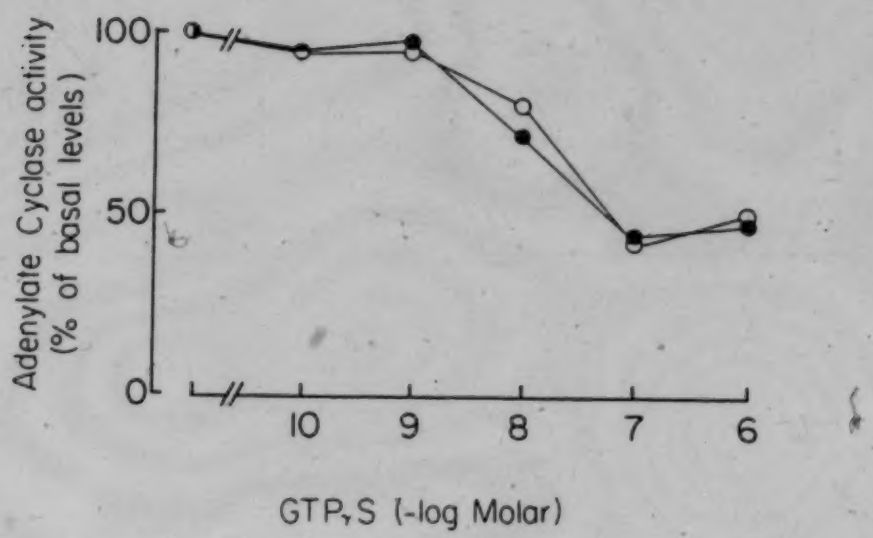


Fig. 57. Effect of GTPγS on cerebellar adenylate cyclase activity at 20°C. The Mg^{2+} concentration was 2 mM. The shaded symbols represent control adenylate cyclase activity. The open symbols represent adenylate cyclase activity of cerebellum treated with supernatant obtained from a lung homogenate (see text for details). The basal activities were 81.2±4.7 and 82.3±1.9 pmoles cyclic AMP/min/mg, respectivley. Each point is the mean of duplicates.

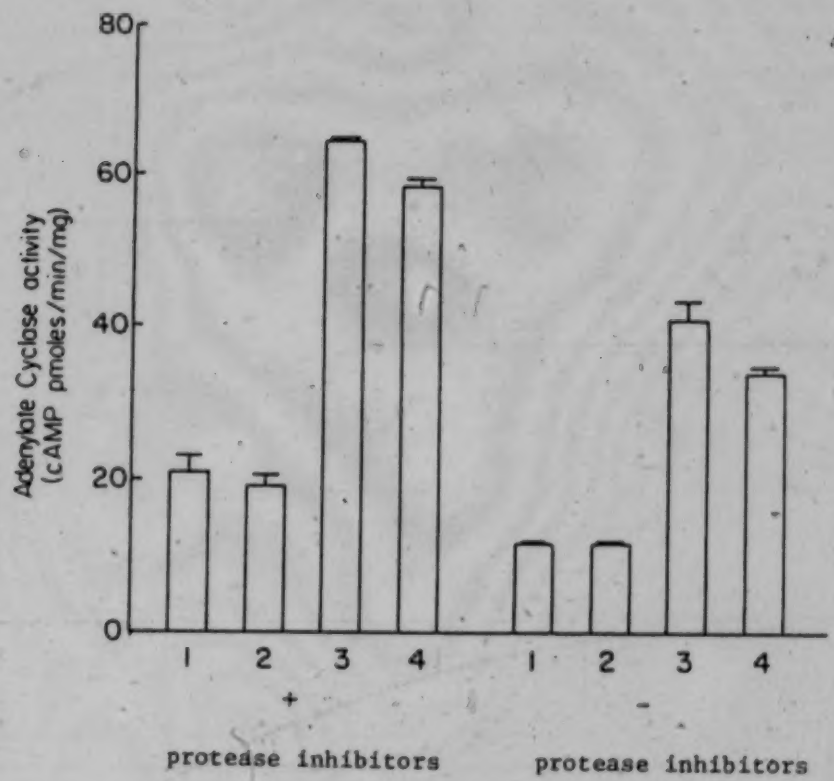


Fig. 58. Effect of protease inhibitors on lung adenylate cyclase activity in the presence of GTPγS (1 nM) at 20°C. The numbers indicate the following: 1 = basal activity; 2 = GTPγS (1 nM); 3 = Forskolin (100 μM); 4 = Forskolin (100 μM) + GTPγS (1 nM). Each bar is the mean±SEM of duplicates.

be related to the low functional status of Ni (as determined with GTPγS) in lung membranes.

Further experiments to determine the effects of a hormonal inhibitor of cerebellar adenylate cyclase (a system where Ni functions are pronounced, see Table 18) were undertaken. This served as a positive control against which the effects of LTD_4 on the lung could be compared. The hormone chosen was GABA (see section 1.2.1.4.7). Furthermore, all subsequent experiments were done at 20°C and 50 μM ATP substrate concentration unless stated otherwise; the former because GTPγS inhibition was greater at lower temperature, and the latter to reduce the production of adenosine in cerebellar adenylate cyclase assays.

4.4.4 Effects of GABA and LTD_4 on cerebellar adenylate cyclase activity.

As shown in Fig. 59 GTPγS-induced inhibition of cerebellar adenylate cyclase activity was not further affected by inclusion of GABA (10 μM) or LTD_4 (10 nM) in the assay. Hence in this system GTPγS cannot substitute for GTP in mediating hormonally-induced inhibition of the enzyme. The crucial role of GTP in mediation of the inhibitory effects of hormones (Rodbell, 1980) is shown in Fig. 60. As shown in Fig. 60, GABA and LTD_4 did not affect cerebellar adenylate cyclase activity in the absence of GTP. However in the presence of GTP, both GABA (10 μM) and LTD_4 (10 nM) inhibited enzyme activity. Maximal

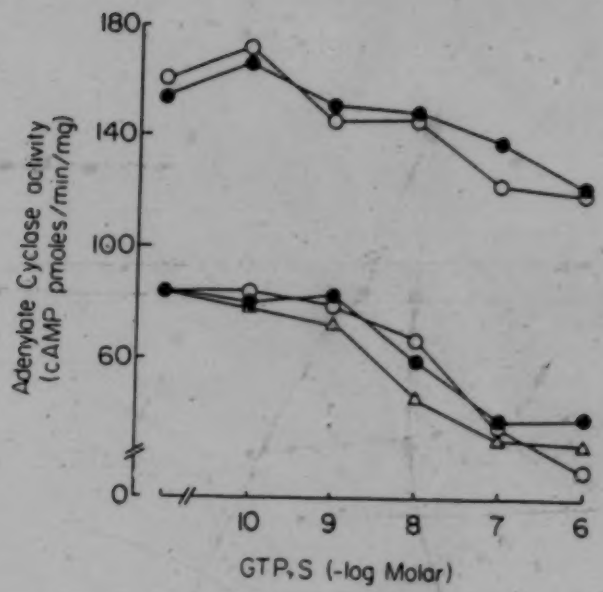


Fig. 59. Effects of GTPγS on cerebellar adenylate cyclase activity in the presence of GABA or LTD_4. The assay was carried out at 20°C in the presence of Mg^{2+} (2 mM). The symbols indicate the following: Basal activity or forskolin (100 μM) stimulated activity (●): LTD_4 (10 nM) (○): or GABA (10 μM) (△). Points are the means of duplicates.

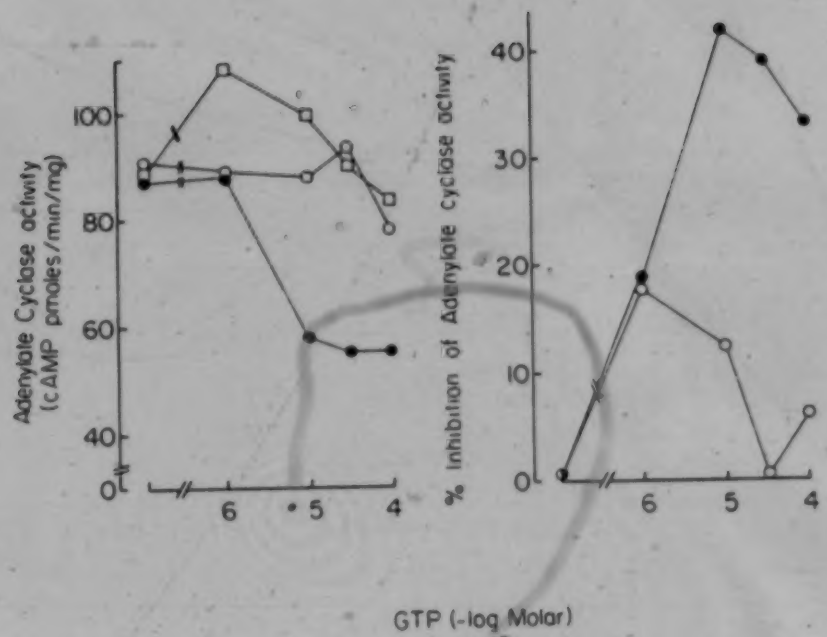


Fig. 60. Effects of LTD_4 and GABA on cerebellar adenylate cyclase activity at 20°C. The effects of GTP are shown in the left panel (□) and of GTP in the presence of LTD_4 (10 nM) (○) or GABA (10 μM) (●). The right panel shows the % inhibition of activity in the presence of GTP alone. Each point is the mean of duplicates.

GABA-induced inhibition of enzyme activity was about 40%.

Inhibition of adenylate cyclase activity occurred maximally at a GTP concentration of 10 μM (Fig. 60). Lower or higher concentrations of GTP tended to reduce the effects of GABA in this and other experiments. LTD_4 (10 nM) also inhibited the enzyme. However, this effect occurred maximally at concentrations of GTP of about 1 μM. Furthermore, the magnitude of inhibition of LTD_4 was small (i.e. less than 20% inhibition of enzyme activity) and as in the lung, problems were encountered with reproducibility of the data between preparations.

The complete concentration-response relationship of GABA in inhibition of cerebellar adenylate cyclase at 20°C, in the presence of GTP (10 μM), is shown in Fig. 61. The maximal effect of GABA (≈35% inhibition) occurred at 10 μM.

In order to demonstrate that the inhibition of adenylate cyclase activity induced by GABA (10 μM) and GTPγS (0.1 μM) was occurring under optimal conditions of temperature and divalent cation concentrations, the experiments shown in Fig. 62 and Table 19 were performed. Maximal inhibition of the enzyme by GABA and GTPγS occurred at 20°C and a low concentration of Mg^{2+} (Fig. 62). Table 19 shows the percentage inhibition of the enzyme by GABA (in the presence of GTP (10 μM) and GTPγS at different concentrations of Mg^{2+} and Mn^{2+} in the assay. Again, maximal inhibition of enzyme activity by GABA (36.3±3.6 n=3) and by GTPγS (57.1±3.0% n=4) occurred at a concentration of 2 mM Mg^{2+}. Higher concentrations of Mg^{2+} and Mn^{2+} reduced GTPγS-induced

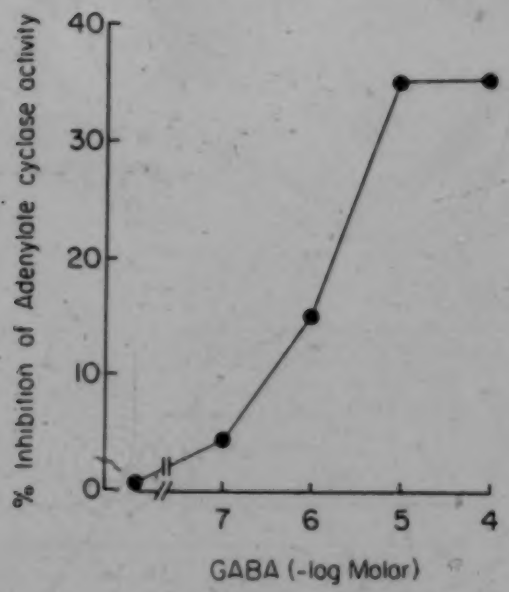


Fig. 61. Inhibition of cerebellar adenylate cyclase activity by GABA at 20°C. GTP (10 μM) was present in all tubes. The Mg^{2+} concentration was 2 mM. Each point is the mean of duplicates.

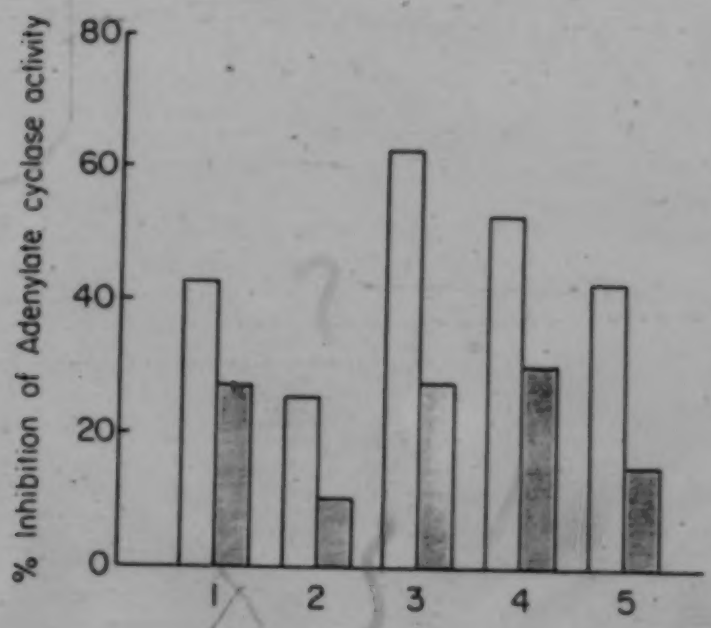


Fig. 62. Inhibition of cerebellar adenylate cyclase activity by GTPγS (clear bars) and GABA (10 μM) in the presence of GTP (10 μM) (shaded bars) under various conditions. The numbers indicate: 1 = Mg^{2+} (2 mM) and 37°C; 2 = Mg^{2+} (10 mM) and 37°C; 3 = Mg^{2+} (0.5 mM) and 20°C; 4 = Mg^{2+} (2 mM) and 20°C; 5 = Mg^{2+} (10 mM) and 20°C. Each bar is the mean of duplicates.

Table 19. % Inhibition of rat cerebellar cyclase basal activity in the presence of Mg^{2+} and Mn^{2+}.

Cation (mM)		GPTγS (0.1 μM)	GABA + GTP (100 μM) (10 μM)
Mg^{2+}	0.5	55.5±10.4%[a]	27.3±0.1%
	2.0	57.1±3.0%	36.3±3.6%
	5.0	62.1±1.2%	17.4±0.1%
	10.0	42.1±0.9%%	15.5±0.3%
Mn^{2+}	1	56.9±0%	10.2±0.4%
	10	37.4±0.9%	Not done

a = mean±SEM (n>2).

inhibition (Table 19).

Therefore, the effects of LTD_4 on cerebellar adenylate cyclase activity were investigated at 20°C and Mg^{2+} (2 mM) in the rest of these experiments.

LTD_4 (10 nM) inhibited enzyme activity in the presence of GTP (Fig. 63). Under the same conditions LTC_4 (10 nM) only slightly inhibited enzyme activity whereas GABA (10 μM) inhibited the enzyme. In this experiment LTD_4 inhibited the enzyme to a greater degree than was observed in other experiments (see Fig. 60). Furthermore, this inhibition occurred at a high concentration of GTP (30 μM). A higher concentration of GTP (100 μM) completely abolished LTD_4 induced inhibition and reduced GABA-mediated inhibition.

As shown in Fig. 64 LTD_4 inhibited enzyme activity in the presence of GTP (10 μM) and slightly stimulated activity in the presence of GTP (100 μM). The concentrations of LTD_4 causing maximal inhibition also caused maximal stimulation (Fig. 64).

In summary, LTD_4 slightly reduced or slightly increased adenylate cyclase activity depending on the GTP concentration in the assay. A high GTP concentration tended to enhance the slight stimulatory effect observed in some experiments. It should be mentioned that in some experiments LTD_4 failed to affect enzyme activity (in the presence of GTP) however GABA-induced inhibition was also relatively low in these experiments.

The lack of reproducibility of LTD_4 effects in the lung and in

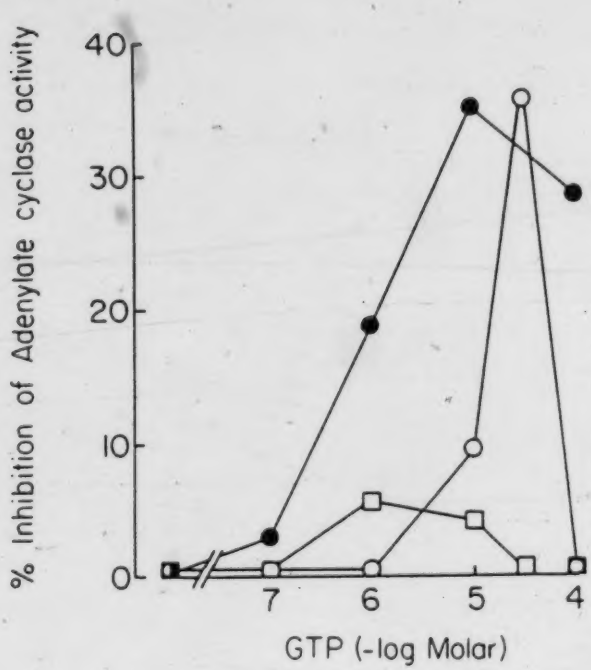


Fig. 63. Percentage inhibition of cerebellar adenylate cyclase activity at 20°C by GABA (10 μM) (●); LTD_4 (10 nM) (○); and LTC_4 (10 nM) (□). The Mg^{2+} concentration was 2 mM. Points are the means of duplicates.

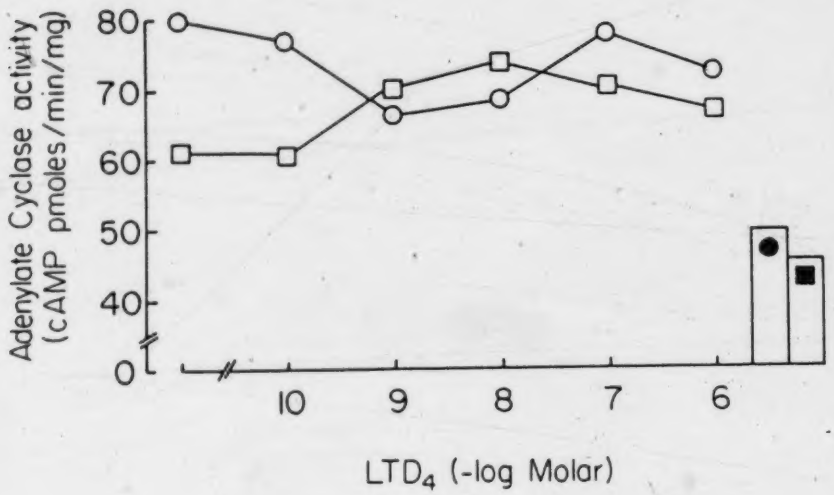


Fig. 64. Effect of LTD_4 on cerebellar adenylate cyclase in the presence of GTP (10 μM) (○) or 100 μM (□). The bars indicate the effect of GABA (10 μM) under both conditions. The assay was carried out at 20°C and Mg^{2+} (2 mM). The points are the mean of duplicates.

cerebellar adenylate cyclase systems was a major problem in these studies. As mentioned earlier, some approaches were undertaken in order to resolve these difficulties. However, the variability persisted and at the end of this work these factors remain unresolved.

5. DISCUSSION

5. Discussion

5.1 Effects of bronchoconstrictors and bronchodilators on normal and sensitized airway tissues:

Challenge of sensitized airway tissues with specific antigen (OA) leads to a prolonged bronchoconstriction in vitro (Schultz-Dale response). The mediators released in this reaction include histamine, LTC_4 and LTD_4 (Fleisch et al. 1982; Section 5.2 of this thesis).

The sensitivity to antigen of sensitized tissues did not result in an enhanced response to the bronchoconstrictor agonists used in this study. Our results indicated that in the absence of inhibitors of AA metabolism there were no appreciable differences between normal and sensitized airways in reactivity and sensitivity to bronchoconstrictors including KCl, histamine, carbachol, LTC_4, LTD_4, U-44069, and A23187. Furthermore, bronchodilator hypoactivity did not exist in sensitized airway tissues since isoproterenol, PGE_2, forskolin, and VIP exhibited similar potency and efficacy on both normal and sensitized airway tissues. Hence in this guinea-pig model of asthma, mere immunological sensitization to OA does not induce bronchoconstrictor hyperreactivity and bronchodilator hypoactivity when these effects are determined in vitro.

It was important to perform the above studies because human bronchial asthma exhibits important characteristics that may differ from animal models of this disease. The results obtained in the guinea pig suggest that mere immunological sensitization per se does

not lead to hyperreactivity and/or hypoactivity and that other factors inherent in human asthma may be deemed to be causatively important. Differences between human asthma and animal models include the following: (1) In human allergic asthma IgE mediates the immediate (type 1) hypersensitivity reaction (Johansson & Bennich, 1982) whereas in the guinea pig, IgG_1 is the predominant immunoglobulin mediating the Schultz-Dale response (Mongar, 1965; Andersson, 1980); (2) Animal studies of reactivity and sensitivity of airway tissues to broncho-constrictors and bronchodilators are often done in vitro, whereas human asthma is studied in vivo; (3) Most animal models of asthma do not have asthma in the same pathological sense as human asthma. In the model studied in this thesis, the animals did not cough or wheeze, did not have congested airways as a consequence of mucus hyper-secretion and did not have inflamed hypertrophied airways as in the typical human asthmatic (Garland, 1984). The animals we studied were simply hypersensitive to antigen. This would imply that the model used may only correlate to atopic asthma in man. In the following discussion the importance of these factors is highlighted.

With regards to IgE and IgG, it has been shown that the guinea pig can produce IgE provided an adjuvant such as $Al(OH)_3$ is used in the sensitization procedure (Mongar, 1965; Andersson, 1980; Andersson, 1982). In guinea pigs sensitized to produce IgE, decreased reactivity to isoproterenol can be demonstrated (Andersson & Bergstrand, 1981). Furthermore, various canine models of asthma that consistently show

hyperreactivity to methacholine and hypoactivity to isoproterenol are IgE producers (Rinard et al. 1979; Antonissen et al. 1980). In guinea pigs sensitized with OA in the presence of Freunds complete adjuvant, hyperreactivity to histamine, 5HT, bradykinin, acetylcholine and $PGF_{2}\alpha$ can be demonstrated (Morcillo et al. 1984). Based on the studies of Andersson (1980), it is possible that IgE may be causative in producing airway hyperreactivity in this guinea-pig model. Evidence that IgE- and IgG-mediated responses are different was obtained by Regal (1984). This author showed that LTs are important mediators of IgG- but not IgE-mediated guinea-pig tracheal contraction. This observation coupled with those of Andersson (1980) and Andersson and Bergstrand (1981) who showed that anti-asthmatic agents differentially affect antigen-induced bronchoconstriction in guinea pigs with high circulating levels of IgE or IgG, seem to indicate that these homocytotropic antibodies may affect airway smooth muscle function and characteristics differentially.

From our results it seems likely that animal models of asthma producing IgG instead of IgE do not exhibit hyperreactivity. This conclusion is also supported by the findings of Brink et al. (1981) who showed that OA sensitized guinea-pig airway tissues do not exhibit hyperreactivity to histamine in vitro.

As mentioned earlier in this discussion, most animal studies of asthmatic hyperreactivity are conducted on isolated tissues. This removes various influences which may be important in the pathogenesis

of airway hyperreactivity. Airway hyperreactivity may arise as a consequence of an enhanced vagal reflex which maybe due to increased sensitivity of irritant receptors in the airways (Leff, 1982; Garland, 1984). This influence is not present if studies are conducted in vitro as are other modulatory influences operating in vivo. Hence their roles cannot be assessed in vitro in order to determine their importance in the pathogenesis of airway hyperreactivity. It is interesting to note that Iwayama et al. (1982) showed airway hyperreactivity to histamine and airway hypoactivity to isoproterenol in OA sensitized guinea pigs (sensitized in a protocol similar to that used in this thesis) in an in vivo study. Furthermore, Brink et al. (1981) showed an increased sensitivity to histamine in guinea pigs after indomethacin pre-treatment. This increased sensitivity was only observed in sensitized animals studied in vivo.

The study by Barnes et al. (1980) demonstrated that in the OA-sensitized and challenged guinea pig there was a decreased β-receptor density and an increased α-receptor density. This animal model exhibited symptoms of asthma and has demonstrable airway hyperreactivity to bronchoconstrictors and hypoactivity to isoproterenol. In this model, the animals had undergone the stress of bronchoconstriction induced by antigen-induced release of mediators in vivo. This may be expected to lead to desensitization of relaxant receptors and/or down regulation (Lefkowitz et al. 1983). Furthermore, modulatory influences in vivo may be operational and with prolonged exposure

to bronchoconstrictors may adapt at lower efficiency of effect. Finally, LTs released in vivo may sensitize airway smooth muscle to the effects of other bronchoconstrictors (section 1.3.3). Therefore, hyperreactivity may arise as a consequence of the stresses of the disease itself (Meurs et al. 1982). Secondly, a major criticism of the hypoactive bronchodilator theory of asthma is that hypoactivity arises as a consequence of long term treatment with bronchodilators such as β-adrenoceptor stimulants (Connolly & Greenacre, 1976). Our results therefore tend to support the above criticisms, since the model we used in this work was immunologically sensitized but unchallenged. This would result in a model that does not exhibit the above characteristics and hence would not exhibit hyperreactivity or hypoactivity of the airways. In other models of asthma, such as naturally allergic canine models used by Rinard et al. (1976), hypoactivity to isoproterenol does exist. However, there may be genetic differences in the canine species which might account for the observed hypoactivity to β-adrenoceptor agonists.

The reduction of tracheal tone by indomethacin (a cyclooxygenase inhibitor), observed in this study, supports and extends other published data (Orehek et al. 1975; Brink et al. 1981). We have also shown that tone can be reduced by a lipoxygenase inhibitor in sensitized and normal tissues. Previous studies have suggested a role for cyclooxygenase products in the maintenance of tracheal tone and in modulation of active contraction of this tissue (Orehek et al. 1975).

These authors proposed that tracheal resting tension is maintained by an intrinsic production of contractile PGs, chiefly $PGF_{2\alpha}$ and PGE_2. Contraction of trachea with histamine or other bronchoconstrictors leads to a production of relaxant PGE_2 which modulates the extent of the contraction. Hence, inhibition of cyclooxygenase would lead to a reduction of tone and to removal of the negative influence of the relaxant PGE_2, thus enhancing contraction. Based on the evidence presently obtained in this study, with histamine, carbachol, and the leukotrienes (Figs. 6 & 7), this scheme was upheld. The fact that NDGA, a lipoxygenase inhibitor, had effects to those exerted by similar indomethacin, is difficult to explain, even though NDGA decreased tone to a lesser extent than indomethacin. It is possible that NDGA at the concentration used exerted some cyclooxygenase inhibiting activity. The reduction of responses to low concentrations of histamine, carbachol, and leukotrienes, and the enhancement of the effects of higher concentrations after treatment of trachea with the inhibitors were similar in both normal and sensitized tissues. This implies similar metabolic pathway for AA in both normal and sensitized tissues. This is in contrast to the effects of high concentrations of the endoperoxide analog (U-44069) after treatment of the tissues with indomethacin (Fig. 8). In this case an enhancement was only observed on normal trachea. U-44069 is a stable analog of the short-lived prostaglandin endoperoxides that are intermediates in the synthesis of PGs from AA via the cyclooxygenase enzyme. U-44069 has been reported

to be platelet aggregatory in man (Menzel et al. 1976), is a bronchoconstrictor in dogs (Wasserman, 1976), and contracts rabbit aorta (Loutzenhiser & Van Breeman, 1981). Its actions resemble TxA_2, a known mediator in human anaphylactic reactions. The lack of enhancement of the effect of U-44069 on sensitized trachea after pretreatment with indomethacin was an unexpected finding, since from the evidence with other bronchoconstrictors we would have expected an enhancement as well. However, U-44069 is not a typical agonist. The C-R curves generated on the trachea were not the normal sigmoid shape, but were biphasic, hence implying an effect at more than one receptor. In addition, low concentrations of this agent exerted similar effects in the presence and absence of indomethacin on normal trachea. The effects of low concentrations were not abolished or reduced, unlike other agonists. Thus U-44069 may be acting differently on trachea. This agent may be activating a "different" cyclooxygenase or utilizing a "different" pool of AA when compared with the other bronchoconstrictors. This "different" enzyme and (or) pool may have been affected by sensitization, and hence no enhancement was observed on sensitized trachea. Support for this hypothesis comes from the work of Sun et al. (1977) who suggested different cyclooxygenases in normal and sensitized tissues. Furthermore, it is known that normal trachea is capable of synthesizing sigificantly greater amounts of prostanoids than sensitized trachea (Burka et al. 1981). Clinically, a diversion of AA metabolism from cyclooxygenase derived to lipoxygenase derived

mediators has been suggested (Yen and Morris, 1981). Thus, our results, to a certain extent, support the pattern of alterations in the activity of AA-metabolizing enzymes as a result of sensitization.

In this work the importance of studying large and small airways becomes apparent. Lung parenchymal strips are representative of the small airways although vascular components are also present (Clayton et al. 1981). However, parenchymal strips as a whole behave more similarly to airway than to vascular smooth muscle (Songsiridej et al. 1982). The differential effects of histamine and carbachol on the trachea and lung parenchyma (Fig. 5) agree with the findings of Drazen & Schneider (1978). These investigators found that carbachol induced a greater response on the trachea than did histamine at equimolar doses, whereas the reverse was true for parenchyma. Further emphasis on differences between large and small airways is demonstrated by the lack of effect of indomethacin, phenidone, and NDGA on the tone of parenchymal strips and the lack of effect of these inhibitors on the contractile responses of the parenchyma to histamine, carbachol, and U-44069 (Fig. 9, Table 9). Although Brink et al. (1981) demonstrated an inhibition of histamine-induced responses on the parenchyma after treatment with indomethacin, we also demonstrated a reduction of the contractile activity of LTD_4 on normal and sensitized parenchyma following treatment with indomethacin, phenidone, and NDGA. This result is in agreement with the work of Piper and Samhoun (1981) who demonstrated a reduction in the contractile activity of LTC_4 and LTD_4

on parenchyma following inhibition of the cyclooxygenase enzyme. Indomethacin and phenidone would inhibit the synthesis of TxA_2, a mediator released from lung tissue following stimulation with leukotrienes and possible other agonists. Hence our results support the concept that leukotrienes exert an indirect effect on the lung parenchyma which is mediated by TxA_2. Although sensitized lung has been reported to produce more cyclooxygenase products than normal lung (Mathe et al. 1977) we did not observe any differenes in the effects of cyclooxygenase and/or lipoxygenase blockade on the contractile activity of LTD_4 on normal and sensitized lung parenchyma.

Adenylate cyclase is intimately linked with a variety of hormone receptors and is one of the links between receptor occupancy and the biological effect (Lefkowitz & Michel, 1983). It can thus be used as a direct index of bronchodilator receptor activation. In our experiments isoproterenol, PGE_2, VIP and forskolin caused significant stimulation of this enzyme. Again, normal and sensitized lungs responded similarly to these relaxants. Hence we can assume that the receptor linkage with the enzyme, and the enzyme itself, are not affected by immunological sensitization.

Although forskolin caused slightly less relaxation of the airways than isoproterenol, the diterpene caused significantly greater stimulation of pulmonary adenylate cyclase than did isoproterenol. Although it has not been conclusively proven, it is widely accepted that certain types of smooth muscle relaxation are mediated by

increased intracellular levels of cyclic AMP (Scheid et al., 1979). Since forskolin stimulates the synthesis of cyclic AMP to a much greater extent than isoproterenol, we would have expected forskolin to cause significantly greater relaxation of the airways than we demonstrated in this work. A possible explanation of this apparent discrepancy is that in the membrane preparation we used the forskolin receptor, possible the catalytic unit of adenylate cyclase (Schmidt et al., 1984), is much more stable and retains its homogeneity even after the somewhat disruptive procedures needed to prepare membranes from the lung. It is known that hormone receptors are extremely labile and it is entirely possible that during membrane preparation part of the receptor pool for a given relaxant would be inactivated. Forskolin is also not selective in terms of stimulation of adenylate cyclase. In our preparations forskolin would stimulate all cyclase units in all cells, whereas isoproterenol would only stimulate β-adrenoceptor linked cyclases. Furthermore, it is possible that airway smooth muscle relaxation may not be directly related to cyclic AMP accumulation which follows activation of the adenylate cyclase. Drugs like nitroglycerine and sodium nitroprusside can relax vascular smooth muscle (Kukovetz et al., 1979) and concomitantly increase levels of cyclic guanosine monophosphate (cyclic GMP) (Kukovertz et al., 1979; Lewicki et al., 1982). Hence there are other candidates besides cyclic AMP that mediate smooth muscle relaxation.

Similar dissociation between airway smooth muscle relaxation and

cyclic AMP accumulation has been observed by Tipton et al. (1981). These authors used isoproterenol to induce a subsensitivity of guinea-pig airway smooth muscle relaxation. However, during the same time period, cyclic AMP accumulation was normal. Hence our results support a discordance between airway smooth muscle relaxation and cyclic AMP levels.

Another point worth considering is that the lung is a very heterogeneous tissue made up of structural, secretory, and contractile cell types. Hence forskolin may activate the adenylate cyclase of non-smooth muscle cell types, whereas isoproterenol, VIP or PGE_2 may be more selective.

Indomethacin, a cyclooxygenase inhibitor, did not affect tracheal relaxation induced by isoproterenol, VIP, PGE_2 or forskolin but did reduce the magnitude of lung parenchymal relaxations to isoproterenol, PGE_2 and forskolin. The data for VIP were too variable to obtain statistical significance. The reduction of bronchodilator-induced relaxations of the lung strip by indomethacin is hard to explain but different hypotheses may be advanced. Indomethacin, by inhibiting the cyclooxygenase enzyme in the lung, would cause a diversion of endogenous AA metabolism to the lipoxygenase pathway (Piper et al., 1979; section 5.2), resulting in enhanced synthesis of potent bronchoconstrictor LTs which would cause the lung strip to be more resistant to a relaxant influence. Indomethacin might also exert a physicochemical action which may affect receptor activation. Since the EC_{50} of each

bronchodilator in relaxing the lung strip did not change after indomethacin-pretreatment, it was assumed that drug binding to the receptor would not have been affected. Indomethacin has been shown by other workers to completely suppress PGE_2-induced bronchodilation in human subjects (Walters et al. 1982). Furthermore, indomethacin has been shown to inhibit the relaxant effects of PGs (Yamaguchi et al. 1976; Burka & Paterson, 1980).

Many drugs including prostacyclin (MacDermot & Barnes, 1980) and VIP (Robberecht et al. 1981) have been shown to activate adenylate cyclase in lung tissue. We have extended these findings to include forskolin. This agent apparently binds to specific sites located on adenylate cyclase itself (Schmidt et al. 1984). We used it as an agent which might reveal differences in adenylate cyclase of lung from normal and sensitized animals. However, forskolin-induced activation of the lung adenylate cyclase was similar in normal and sensitized animals. Hence, we assumed that the adenylate cyclase systems of normal and sensitized lungs were identical. Furthermore, indomethacin-pretreatment did not significantly affect activation of cyclase induced by forskolin, isoproterenol, PGE_2 or VIP, in normal or sensitized lungs.

Finally, Burka (1983a,b) has shown that isoproterenol, forskolin and PGE_2, in order of potency, can inhibit tracheal contractions induced by OA. Higher concentrations of these agents were needed to inhibit A23187-induced contractions to a similar degree as OA-induced

contractions. Furthermore, low concentrations of these agents enhanced tracheal contraction induced by A23187 in sensitized trachea but not on normal trachea where only inhibition was observed. These agents activate adenylate cyclase and cyclic AMP accumulation which may lead to inhibition of mediator release (Lichtenstein et al., 1979). The differential effects of these bronchodilators on normal and sensitized trachea suggests an alteration of cyclic AMP turnover as a consequence of sensitization.

In summary, the OA sensitized guinea-pig model of asthma does not exhibit easily discernible differences in airway reactivity to bronchoconstrictors or to bronchodilators when studied in vitro.

5.2 AA metabolism in guinea-pig airways. Role of calcium:

In these studies we investigated the nature of the mediators released during stimulation with A23187, OA and AA. We also investigated the effect of Ca^{2+} on AA-induced contraction of the airways. This process depends on initial synthesis of LTs from AA via the lipoxygenase pathway and subsequent contraction of the tissue induced by synthesized LTs. Both synthesis of LTs and contraction of the airways are Ca^{2+} dependent processes. Our aim was to see which of these two processes was more dependent on Ca^{2+}.

Stimulation of AA metabolism by antigen (OA) and A23187, and addition of exogenous AA to indomethacin-pretreated airway tissues, resulted in a prolonged contraction. The contractions were reduced by

NDGA, a lipoxygenase enzyme inhibitor. Furthermore, mepacrine, a phospholipase A_2 inhibitor, did not reduce exogenous AA-induced contractions of the airways but reduced contractions induced by OA and A23187. Finally, analysis of the bath fluid by HPLC after stimulation with OA, A23187, or AA, demonstrated the presence of LTC_4 and LTD_4. The identity of these substances was further substantiated by the biological activity they exhibited on the guinea-pig ileum and lung strip and by UV spectra comparisons with authentic LTC_4 and LTD_4.

Quantitative bioassay of the amounts of LT-like activity (confirmed by radioimmunoassay by Burka, 1985) was conducted on the guinea-pig ileum. The results with indomethacin are interesting. We expected that inhibiting the cyclooxygenase pathway would enhance release since this has been observed earlier in other LT generating system (Piper et al. 1979). On the basis of the contractile response of the trachea to OA and A23187 this would seem to have occurred, since we had enhancement of the contraction after indomethacin pretreatment. However, the actual amounts released, as determined by bioassay could not possibly explain the enhancement of contraction as being due to increased release of the mediator. The observed enhancement of contractions may be due to the removal of the negative influence of relaxant PGE_2 which is continuously synthesized by the trachea (Orehek et al. 1975; Burka et al. 1981). PGE_2 exerts a modulatory influence on contractile stimuli acting on the trachea (Orehek et al. 1975). Indomethacin would reduce the synthesis of this

mediator and hence larger contractions for the same or lesser amounts of LTC_4 (or other stimuli) might ensure (section 5.1). Inhibition of SRS-A release by indomethacin has also been observed in rat peritoneal cells (Burka & Flower, 1978; Bach et al. 1977) but may reflect inhibition of calcium flux by indomethacin (Burka & Flower, 1974). The possibility that indomethacin treatment of the trachea may be stimulating the synthesis of another contractile mediator, which we have not yet identified, must not be discounted. AA did not further affect contractions or release beyond that obtained in the presence of indomethacin alone. This may reflect possible saturation of the system at its normal state (i.e. in the absence of AA). Hence further substrate additions would achieve no further effect. It is also conceivable that exogenous AA is not available to the lipoxygenase enzymes during antigen- and ionophore-induced release. This possibility is unlikely since AA induces contraction of the trachea in the presence of a cyclooxygenase inhibitor and the contractions are abolished by lipoxygenase inhibitors.

However, Kuehl et al. (1984) have reviewed evidence that in some systems, addition of exogenous AA would only result in LT synthesis if a stimulus such as A23187 is also present. In the above systems it is conceivable that exogenous AA may displace endogenous bound AA and thus be available for lipoxygenation.

AA is metabolised by lung tissue into contractile cyclooxygenase products (Hamberg & Samuelsson, 1976). Guinea-pig trachea has been

shown to release PGs and TxA_2 after challenge with OA, A23187 and AA (Burka et al. 1981). AA can also be metabolized to lipoxygenase products by guinea-pig airways (Piper et al. 1979; Mitchell & Denborough, 1980; Yen, 1981). In this work we have demonstrated that AA is metabolized by indomethacin-treated guinea-pig airway tissues to LTC_4 and LTD_4. This establishes the inherent capacity of the airways to produce these potent bronchoconstrictors. It is not essential for a stimulus such as A23187 or OA to be present for AA to be metabolized to the above products.

The magnitude of AA-induced contraction was greater on trachea than on lung parenchyma. This is significant especially because the small airways are particularly sensitive to leukotrienes (Drazen et al. 1980). Lung parenchymal strips in the absence of indomethacin contracted to a level equivalent to that observed on the trachea. Subsequent addition of indomethacin partially inhibited the contraction to AA (results not shown). Furthermore, the LTs exert both direct and indirect contractile effects on the lung strip, the latter action being mediated by TXA_2 (Piper & Samhoun, 1981). In our experiments this latter component was abolished by indomethacin. Our results are in agreement with those of Mitchell and Denborough (1980) and Yen (1981).

The effects of mepacrine support the evidence that exogenous AA is metabolized to LT, since the magnitude of OA- and A23187-induced contractions was reduced whereas exogenous AA was unaffected. In the

experiments conducted on mepacrine-treated tissues, AA probably had an additive effect with antigen or ionophore on the trachea. An additive effect of exogenous AA on the lung strip was not observed even though mepacrine appeared to inhibit phospholipase A_2, based on the criteria that the magnitude of antigen- and ionophore-induced contractions was significantly reduced.

The two inhibitors of lipoxygenase, NDGA and phenidone, both markedly reduced the effects and inhibited release by all three stimuli we used. This supported the observations that contractions induced by AA, OA and A23187 are partly a result of leukotriene synthesis.

The presence of the lipoxygenase enzyme in lung tissue has been confirmed for both guinea-pig lung (Hamberg & Samuelsson, 1974) and human foetal lung (Saeed & Mitchell, 1982). It has also been observed that the basal levels of gluthathione-S-transferase, the enzyme that converts LTA_4 to LTC_4, are higher in normal lung than in sensitized lung (Morris et al. 1982). However, no significant difference in leukotriene production between normal and sensitized tissues, when activated with AA, was observed in the present experiments. This is in contrast to the results of Piper and Seale (1979) who observed a greater production of SRS-A from sensitized lung than from normal lung. The use of fragments stimulated with A23187 by these authors may account for the difference from our results using parenchymal strips stimulated with AA while under tension in organ baths.

Furthermore, our methods of purification of the released mediators differed.

Finally, we have shown that addition of substrate (i.e. AA) to indomethacin-treated guinea-pig airways induced synthesis of similar lipoxygenase metabolites to those produced upon activation with ionophore or antigen. In some cells, such as human polymorphonuclear leukocytes, the AA products formed are dependent on the stimulus. AA stimulation leads to very little lipoxygenase product formation, whereas combination of AA with A23187 leads to the production of significant amounts of 5-hydroeicosatetraenoic acid and LTB_4 (Borgeat & Samuelsson, 1979). This does not appear to be the situation in the indomethacin-treated airways. Mere addition of substrate is an adequate stimulus for activation.

With regards the nature of Ca^{2+} influence on AA-induced contractions of airway tissues, the present experiments demonstrated that AA-induced contractions of guinea-pig airways are calcium-dependent. AA-induced contractions of trachea were reduced after 30 min incubation in Ca^{2+}-free KHS. Similarly, LTC_4-induced contractions were reduced under the same conditions. Since we have earlier shown that AA is metabolized by guinea-pig trachea to LTC_4 and LTD_4, the above results were expected. The 30 min incubation in zero-calcium medium is sufficient to remove all free extracellular calcium ions from the trachea (Creese & Denborough, 1981). In our experiments we observed that lung parenchyma was more resistant to extracellular

calcium removal since the contractions to AA were unchanged in Ca^{2+}-free KHS from those of control. This calcium resistance of lung parenchyma has also been observed by other authors (Burka, 1984a; Weichman et al. 1983; Hedman and Andersson, 1982). The heterogeneity of lung parenchyma, relative to the trachea, probably contributes to this calcium resistance.

Although calcium is important for AA-induced contractions of airways, addition of excess levels of this ion does not further increase the response since we failed to see increased responses in KHS containing four-fold higher levels of calcium.

Addition of lanthanum or EDTA completely abolished contractions to AA and LTC_4 in Ca^{2+}-free KHS. This occurred on both trachea and parenchyma. Lanthanum displaces calcium from superficially bound sites on cell membranes (Pearce & White, 1981; Freeman & Daniel, 1983). This element had no effect in normal KHS which agrees with earlier results where lanthanum had no effect on antigen- or A23187-induced contractions of airways in normal KHS (Burka, 1983 c). Lanthanum eliminated the residual contraction of trachea to AA and LTC_4, and of parenchymal contraction to AA and LTC_4 that was observed in Ca^{2+}-free KHS. This is evidence for loosely-bound membrane pools of calcium that can participate in airway contraction to AA and LTC_4. Lanthanum displaces these pools and thus renders them unavailable for contraction. The results with EDTA also support this concept. EDTA chelates extracellular calcium and can thus completely deprive airway

tissues of all extracellular calcium.

TMB-8, the intracellular calcium antagonist, which acts by stabilization of calcium ions to intracellular binding sites (Malagodi& Chiou, 1974) was very effective in completely inhibiting AA-induced contractions of the airways. This action of TMB-8 occurred both in the presence and absence of extracellular calcium. Weichman et al. (1983) have shown that TMB-8 completely abolishes LTD_4-induced contractions of guinea-pig trachea and parenchyma and Burka (1983c) showed that TMB-8 inhibits OA and A23187-induced contractions of guinea-pig airways. Furthermore, we have shown that trifluoperazine, an intracellular calmodulin antagonist, and EGTA, a calcium chelator, were very effective in inhibiting antigen- and A23187-induced contraction of trachea and parenchyma (Burka, 1984).

The above results indicate that calcium plays a vital intracellular role in AA-induced contractions. Furthermore, calcium from a lanthanum-sensitive extracellular compartment probably contributes to the overall process of AA-induced contraction.

What is the main role of calcium in AA-induced contraction of the airways? To shed light on this question we used two calcium entry blockers, verapamil and nitrendipine. These drugs inhibit the influx of calcium ions through voltage-dependent channels (Triggle, 1983). In our experiments verapamil and nitrendipine inhibited AA-induced contractions of the airways but did not affect LT-induced contractions (Figs 31 & 34; Burka, 1983c; Weichman et al. 1983). Thus, verapamil

and nitrendipine appear to exert their inhibitory effects by preventing the synthesis of LTs from AA and not by affecting smooth muscle contraction induced by LTs. This concept agrees with the work of Patel (1981 a, b) who demonstrated that nifedipine and verapamil inhibited exercise-induced asthma by inhibiting mediator release, since they did not have any effect on bronchoconstriction induced by histamine or methacholine. The 5-lipoxygenase enzyme responsible for the oxidation of AA to 5-HPETE, the precursor for LT synthesis, is calcium-dependent (Jakschik & Lee, 1980; Furukawa et al. 1984) and it is therefore highly probable that this is where calcium is acting in this study.

Nitrendipine was effective in inhibiting the tracheal and parenchymal contractions obtained upon re-addition of calcium to tissues in Ca^{2+}-free KHS plus lanthanum. This effect of nitrendipine further demonstrates the importance of calcium in AA-induced contractions of the airways and provides evidence that calcium is required to cross the cell membrane to an intracellular site of action.

The effects of calcium entry blockers on airway smooth muscle generally occur at much high concentrations than needed for vascular smooth muscle (Triggle, 1983; Lefer et al. 1984). This tissue selectivity is still poorly understood and distracts from the concept that calcium entry blockers may be useful therapeutic agents in asthma since they may significantly affect cardiovascular function at doses

that inhibit asthmatic bronchoconstriction.

5.3 Effects of LTD_4 on lung and cerebellar adenylate cyclase:

As mentioned in the Results, the effects of LTD_4 on adenylate cyclase activity, were complicated by a lack of reproducibility apparently beyond experimental control. Smooth muscle cyclases are notoriously difficult to study because of the variability of measurable hormone-induced effects (Muller, 1985). Hormone-receptor linkage to intracellular effectors, such as adenylate cyclase, is easily perturbed by slight changes in homogenization conditions (Rinard & Jensen, 1981; Muller, 1985).

In this dissertation, we used guinea-pig lung membranes to study the effects of LTD_4 on adenylate cyclase. The enzyme was studied under various conditions. These included low temperature (20°C) and high temperature (37°C), the use of forskolin and isoproterenol to stimulate the enzyme to a higher activity, the use of GTP and GTPγS, the use of various concentrations of divalent cations (Mg^{2+}, Mn^{2+} and Ca^{2+}), and the use of Na^{+} to optimize inhibition. The rationale for the above approaches is described as appropriate in the Results and here in the Discussion.

Guinea-pig lung is a tissue that contains a significant proportion of airway smooth muscle cells (terminal bronchioles) and, to a lesser degree, vascular smooth muscle cells (MacDermot & Barnes, 1980; Songsiridej et al. 1982). In addition, there are other non-contractile cellular elements in the lung. There is no doubt that LTD_4 contracts

lung tissue by an effect ultimately exerted on smooth muscle cells. This contractile action can be characterized as a whole in terms of binding of LTD_4 to specific receptors in the lung (Pong & DeHaven, 1983) and in terms of calcium-dependence of the contractile effect (see section 5.2).

The link between binding of LTD_4 to its receptors and calcium mobilization in the vicinity of contractile proteins is unknown. The hypothesis followed in this dissertation was that LTD_4 receptors are negatively linked to adenylate cyclase and occupation of the LTD_4 receptor ultimately leads to reduced cyclic AMP synthesis with consequent effects on calcium mobilization, leading to contraction.

The above scheme of events is supported by studies of various facets of this system. These include: (1) studies of LTD_4 binding to receptors and regulation of such binding by guanine nucleotides, divalent and monovalent cations (see section 1.2.1.4.6). This implies a negative influence on adenylate cyclase activity (Rodbell, 1980; Jakobs et al. 1984); (2) studies of calcium-dependency of smooth muscle contraction induced by constrictors including LTD_4 (Stull and Sanford, 1981; Middleton, 1984; Rasmussen & Barret, 1984); (3) studies of the link between levels of cyclic AMP and levels of calcium in the intracellular compartment. This is discussed below.

Substantial evidence exists to suggest that cyclic AMP and calcium may function as intermediates in pharmaco-mechanical and stimulus-secretion coupling in various cells. With regards to the

LTs, it is likely that the contractile effect of LTs on the airways is mainly due to release of intracellular Ca^{2+} (Weichman & Tucker, 1984; Raeburn & Rodger, 1984). Furthermore, Ca^{2+} release by subcellular fractions of smooth muscle is increased in parallel with a reduction of cyclic AMP levels (Hedman & Andersson, 1982). Hence this would lead to a contractile effect (Scheid et al. 1979). Furthermore, cyclic nucleotides regulate Ca^{2+} sensitivity of secretory processes. An increase in cyclic AMP inhibits secretion, primarily by reducing intracellular Ca^{2+} concentrations (Knight & Scrutton, 1984). More direct evidence of the involvement of Ns and Ni in regulation of intracellular Ca^{2+} levels was obtained by Molski et al. (1984). These authors showed that inhibition of Ni functions with pertussis toxin leads to a reduction of the LTB_4-induced increase in membrane permeability to Ca^{2+}. Finally, cytoplasmic Ca^{2+} levels in platelets is increased after stimulation with thrombin (Zavoico & Feinstein, 1984). Thrombin inhibits platelet adenylate cyclase (K. Jakobs, personal communication). Hence cyclic AMP serves as an inhibitory second messenger that antagonizes the mobilization of Ca^{2+}. Reduction of cyclic AMP levels by inhibitors of adenylate cyclase would lead to Ca^{2+} release from intracellular stores and/or Ca^{2+} uptake and consequent contractile and secretory events.

Hence, in this work, the aim was to demonstrate or exclude an effect of LTD_4 on adenylate cyclase. Taking the previously mentioned factors into account, it was necessary to demonstrate that LTs can

bind to lung membranes used for cyclase assays. This was demonstrated in all experiments performed with this aim in mind. Thus we were assured that the lack of positive results in a particular experiment was not due to the fact that LTD_4 was not binding to its receptors in the membrane preparation. Other explanations must be sought and amongst these would be a nonfunctional linkage of the receptor with one or more of the components of adenylate cyclase. Furthermore, it was important to demonstrate that the various components of adenylate cyclase are functional. Our preparations responded to various hormones, guanine nucleotides, divalent cations and forskolin. This implied that the hormone receptors, guanine nucleotide binding proteins (Ns and Ni) and the catalytic unit of the enzyme were intact and functional. To a certain degree it also implied that these units could interact with each other in the membrane prepared from whole tissue. However, it did not imply that such an interaction was exactly as found in the intact tissue in vivo.

Adenylate cyclase activity of lung membranes had an 8-fold lower specific activity than cerebellar membranes. This may be reflection of the relative homogeneity of cell types in the cerebellum when compared to the lung. Furthermore, a more important difference between these two tissues was the fact that GTPγS inhibited cerebellar cyclase activity to a much greater degree than lung cyclase activity. The process of inhibition of cyclase activity was probably mediated by Ni. In this study various characteristics (Jakobs et al. 1984) of Ni

mediated inhibition were tested and in all cases the system responded typically. Hence the inhibitory effect was greater at a low temperature (20°C) compared to 37°C. Furthermore, inhibition was reduced by high Mg^{2+} and Mn^{2+} concentrations. Since GTPγS inhibits adenylate cyclase via Ni, it was logical to conclude that Ni existed in a quantitatively and/or qualitatively greater degree in the cerebellum than in the lung. This is reflected by the fact that LTD_4-induced inhibition was much greater in the cerebellum than in the lung. Furthermore, adenosine and GABA inhibited cerebellar adenylate cyclase activity markedly. GABA did not affect lung cyclase activity to any significant degree (data not shown). Unfortunately, there is no known hormone that will inhibit lung adenylate cyclase activity in a GTP-dependent fashion. Hence, the effects of LTD_4 on lung cyclase could not be compared to another hormone in this tissue. As mentioned earlier, the lack of consistent data distracts from the significance of these findings. LTD_4 inhibited lung cyclase activity with the following characteristics: (1) The inhibition by LTD_4 was small and between 10-15% of the control level. However, on some occasions relatively greater effects (about 60-80% inhibition of enzyme activity) were obtained. The fact that these greater effects occurred reproducibly, though, as a whole, rarely, is significant. Obviously, an unknown factor is responsible and sometimes this factor was permissive for the greater effect to occur. (2) The inhibition by LTD_4 did not exhibit a classical concentration-response relationship.

This observation is true for both lung and cerebellar cyclase systems. However, it was noted that the maximum effect of LTD_4 occurred at low concentrations about 10-100 nM in the lung and at about 10 nM in the cerebellum. Lower or higher concentrations of LTD_4 did not inhibit the enzyme to the same degree. Furthermore, in some experiments (Fig.45), high concentrations of Na^+ optimized the inhibitory response to LTD_4 in the lung. Na^+ reduces binding of LTD_4 to its receptor (see section 1.2.1.4.6) and has been shown to optimize hormone-induced inhibition in some systems. The fact that this occurred in the lung adds further support to the argument that LTD_4 inhibits lung adenylate cyclase. Na^+ did not affect cerebellar cyclase inhibition by LTD_4, GABA, or GTPγS (data not shown). (3) LTD_4 or GABA did not affect cerebellar adenylate cyclase activity in the absence of GTP or in the presence of GTPγS. Effects were only measured in the presence of GTP, the maximum effect occurring at GTP (10 μM); (4) An important observation was the fact that LTC_4 did not inhibit cerebellar adenylate cyclase under conditions where LTD_4 and GABA inhibited the enzyme in the presence of GTP. Secondly, in the presence of high concentrations of GTP (>50 μM), LTD_4 tended to slightly stimulate cerebellar enzyme activity. Under this condition, the concentrations of LTD_4 that caused the most stimulation were the same that caused the maximum inhibition (about 10 nM).

In the lung, LTD_4 (10 nM) slightly stimulated enzyme activity in the presence of Mn^{2+}. However, in the presence of GTP (10 μM), LTD_4

(10 nM) slightly inhibited the enzyme. The above evidence further indicated that LTD_4 effects cannot be nonspecific. The effects although small, occurred consistently at a given concentration. Similar observations have been made regarding the inhibitory effects of other hormones on other systems. Abramowitz & Campbell (1983) showed that [D-Ala^2, Met^5] enkaphalin amide (Da-ENK) inhibited rabbit luteal adenylate cyclase activity in a GTP dependent fashion. Da-ENK did not inhibit the enzyme in the presence of Gpp(NH)p, a nonhydrolysable analog of GTP. The extent of inhibition by Da-ENK was about 16-24%, which is small, relative to other studies. Our results with LTD_4 in the lung are comparable with regards to the above features.

Webster & Olsson (1981) demonstrated that adenosine inhibited canine cardiac adenylate cyclase activity in the presence of GTP. In the presence of Mg^{2+}, stimulatory effects were measured. However, addition of Mn^{2+} (0.5 mM) allowed adenosine to inhibit the enzyme. Again, qualitatively similar effects were measured in this work using LTD_4 and lung adenylate cyclase.

As mentioned earlier, LTD_4 effects were small. The various approaches tried in this work, to maximize the inhibitory effect were futile. The work with GTPγS demonstrates that Ni is not strongly functional in the lung preparation. It is conceivable that the heterogeneity of lung cells contribute to the low Ni-mediated effects. Ni, if present in physiologically effective concentrations in a given cell type, would be diluted by other cell types not containing Ni.

Hence small effects would result. This hypothesis might be proven if lung cells were dispersed and each cell type studied individually. Hence one of the major conclusions of this work is that LTD_4 effects in the lung should be investigated on homogenous cellular preparations of lung tissue. Furthermore, the biphasic effects of LTD_4 on the cyclase systems studied seem to imply that LTD_4 may bimodally regulate adenylate cyclase activity probably via receptor subtypes. The lack of specific agonists for LT receptors makes it difficult to test such a hypothesis. It is well known in pharmacology that receptor subtypes are a fact for various hormone classes. Receptors that bimodally regulate adenylate cyclase activity include adenosine Ra and Ri receptors, α- and β-adrenoceptors and $GABA_A$ and $GABA_B$ receptors. It is conceivable that such receptors exist for LTs and their clarification awaits the development of specific agonists and antagonists. Recent work from other laboratories lends support to the observations made in this work. Nicosia et al. (unpublished observations) also found that LTD_4 reproducibly inhibits lung adenylate cyclase activity by about 10%. Furthermore, Mong and his coworkers (unpublished observations) have also observed similar effects. These workers also showed that LTD_4 stimulates adenylate cyclase activity in whole lung fragments in the presence of low concentrations of forskolin.

In summary, LTD_4 bimodally regulated adenylate cyclase activity in lung and cerebellum, in a GTP dependent fashion. The preliminary evidence presented here supports the above conclusion. However, a

different approach is needed to substantiate the above claim. Such an approach must utilize homogenous cellular preparations of lung tissue. With regards to airway smooth muscle cells, there are a variety of as yet undefined technological difficulties in obtaining hormone-responsive adenylate cyclase.

The physiological significance of the above findings are as yet unknown. However, it is conceivable that lowering of cyclic AMP levels in smooth muscle cells may lead to a reciprocal effect on calcium levels. Furthermore, the above hypothesis should not distract from a newer viewpoint that calcium mobilizing receptors have been shown to exert effects on yet another intracellular messenger, namely, phosphatidylinositol and its "hormone-receptor interaction" stimulated hydrolysis (Salmon & Honeyman, 1980; Berridge, 1981, 1984; Baron et al. 1984).

BIBLIOGRAPHY

Abramowitz J, Campbell AR (1983) Enkephalin-mediated inhibition of forskolin stimulated rabbit luteal adenylyl cyclase activity. Biochem Biophys Res Commun 116: 574-580

Adams GK, Lichtenstein L (1979) In vitro studies of antigen-induced bronchospasm: effect of antihistamine and SRS-A antagonist on response of sensitized guinea pig and human airways to antigen. J Immunol 122: 555-562

Adcock JJ, Garland LG (1980) A possible role for lipoxygenase products as regulators of airway smooth muscle reactivity. Br J Pharmac 69: 167-169

Aktories K, Schultz G, Jakobs KH (1979) Inhibition of hamster fat cell adenylate cyclase by prostaglandin E_1 and epinephrine: requirement for GTP and sodium ions. Febs Letts 107: 100-104

Aktories K, Schultz G, Jakobs KH (1981) Na^+ amplifies adenosine receptor-mediated inhibition of adipocyte adenylate cyclase. European J Pharmacol 71: 157-160

Alm PE, Bloom GD (1982) Cyclic nucleotides in histamine release from mast cells - a reevaluation. Life Sciences 30: 213-218

Anderson ME, Allison RD, Meister A (1982) Interconversion of leukotrienes catalysed by purified γ-glutamyltranspeptidase: concomitant formation of leukotriene D_4 and γ-glutamyl amino acids. Proc Natl Acad Sci USA 79: 1088-1091

Andersson P (1980) Antigen-induced bronchial anaphylaxis in actively sensitized guinea-pigs: patterns of response in relation to immunization regimen. Allergy 35: 65-71

Andersson P (1982) Effect of inhibitors of anaphylactic mediators in two models of bronchial-anaphylaxis in anaesthetized guinea-pigs. Br J Pharmac 77: 301-307

Andersson P, Bergstrand H (1981) Antigen-induced bronchial anaphylaxis in actively sensitized guinea-pigs: effect of long-term treatment with sodium cromoglycate and aminophylline. Br J Pharmac 74: 601-609

Andersson RGG, Nilsson KD (1977) Role of cyclic nucleotides metabolism and mechanical activity in smooth muscle. In: Biochemistry of smooth muscle. Ed Stephens SNL. University Park Press, Baltimore pp. 263-291

Antonissen LA, Mitchell RW, Kroeger EA, Kepron W, Stephens NL, Bergen J (1980) Histamine pharmacology in airway smooth muscle from a canine model of asthma. J Pharmacol Exp Ther 213: 150-155

Assem ESK, Schild HO (1969) Inhibition by sympathomimetic amines of histamine release by antigen in passively sensitized human lung. Nature 224: 1028-1029

Augstein J, Farmer JB, Lee TB, Sheard P, Tattersall ML (1973) Selective inhibitor of slow reacting substance of anaphylaxis. Nature (New Biol) 245: 215-217

Austen KF, Orange RP (1975) Bronchial asthma: the possible role of the chemical mediators of immediate hypersensitivity in the pathogenesis of subacute chronic disease. Am Rev Respir Dis 112: 423-436

Bach MK (1982) Role of leukotrienes as mediators of allergic reactions. Afr J Clin Exp Immunol 3: 89-102

Bach MK, Brashler JR, Gorman RR (1977) On the structure of SRS-A; evidence of biosynthesis from arachidonic acid. Prostaglandins 14: 21-38

Bach MK, Brashler JR, Smith HW, Fitzpatrick FA, Sun FF, McGuire JC (1982) 6,9-Deepoxy-6,9(phenylimino)-$\Delta^{6,8}$-prostaglandin I, (U-60257), A new inhibitor of leukotriene C and D synthesis: in vitro studies. Prostaglandins 23: 759-771

Baer HP (1974) Cyclic nucleotides and smooth muscle. Adv Cyclic Nucleotide Res 4: 195-237

Baer HP (1975) Measurement of adenyl cyclase and cyclic AMP in smooth muscle. In Methods in Pharmacology. Ed Daniel EE, Paton DM pp. 593-611. New York: Plenum Press

Bar-Yishoy E, Godfrey S (1984) Mechanism of exercise-induced asthma. Lung 162: 195-204

Barnes PJ (1983) Calcium-channel blockers and asthma. Thorax 38: 481-485

Barnes PJ, Basbaum CB, Nadel JA, Roberts JM (1982) Localization of beta-adrenoceptors in mammalian lung by light microscopic autoradiography. Nature 299: 444-447

Barnes PJ, Dollery CT, MacDermot J (1980) Increased pulmonary α-adrenergic and reduced β-adrenergic receptors in experimental asthma. Nature 285: 569-571

Barnes PJ, Nadel JA, Roberts JM, Basbaum CB (1983) Muscarinic receptors in lung and trachea: autoradiographic localization using quinuclidinyl benzilate. European J Pharmacol 86: 103-106

Baron CB, Cunningham M, Strauss JF, Coburn RF (1984) Pharmaco-mechanical coupling in smooth muscle may involve phosphatidyl-inositol metabolism. Proc Natl Acad Sci USA 81: 6899-6903

Benveniste J (1974) Platelet-activating factor, a new mediator of anaphylaxis and immune complex deposition from rabbit and human basophils. Nature 249: 581-584

Berridge MJ (1981) Phosphatidylinositol hydrolysis and calcium signaling. In advances in cyclic nucleotide research, Vol. 14. Ed by Drumont JE, Greengard P, Robison GA. Raven Press, New York, pp. 289-299

Berridge M (1984) Inositol triphosphate and diacylglyceral as second messengers. Biochem J 220: 345-350

Berridge MJ, Irvine RF (1984) Inositol triphosphate, a novel second messenger in cellular signal transduction. Nature 312: 315-321

Bianco S, Griffin JP, Kamburoff PH, Prime FJ (1974) Prevention of exercise-induced asthma by indomarin. Br Med J 4: 18-20

Biggs DF (1984) Direct and indirect actions of histamine on airway smooth muscle in guinea pigs. Can J Physiol Pharmacol 62: 727-733

Billah MM, Lapetina E, Cuatrecasos P (1980) Phospholipase A_2 and phospholipase C activities of platelets. J Biol Chem 255: 10227-10232

Blackwell GJ, Burka JF, Flower RJ, Torkington P (1980) On the preparation of highly purified slow reacting substance of anaphylaxis (SRS-A) from biological extracts. Br J Pharmac 68: 33-46

Blackwell GJ, Carnuccio R, diRosa M, Flowers RJ, Parente L, Persico P (1980) Macrocortin: a polypeptide causing the antiphospholipase effects of glucocorticoids. Nature 287: 147-149

Blackwell GJ, Flower RJ (1978) 1-phenyl-3-pyrazolidone: an inhibitor of cyclo-oxygenase and lipoxygenase pathways in lung and platelets. Prostaglandins 16: 417-425

Blackwell GJ, Flower RJ (1978) 1-phenyl-3-pysazolidone: an inhibitor of arachidonate oxidation in lungs and platelets. Br J Pharmac 63: 360

Blackwell GJ, Flower RJ, Nijkamp FP, Vane JR (1978) Phospholipase A_2 activity of guinea pig isolated perfused lungs: stimulation and inhibition by anti-inflammatory steroids. Br J Pharmac 62: 79-90

Bograni S, Folco GC, Razzetti R, Schiantarelli P (1983) B_2-adrenoceptor blockade is the basis of guinea-pig bronchial hyperresponsiveness to leukotriene C_4 and other agonists. Br J Pharmac 79: 839-848

Boot JR, Cockerill AF, Dawson W, Mallen DNB, Osborne DJ (1978) Modification of prostaglandin and thromboxane release by immunological sensitization and successive immunological challenges from guinea-pig lung. Int Archs Allergy Appl Immuno 57: 159-164

Borgeat P, Samuelsson B (1979a) Transformation of arachidonic acid by rabbit polymorphonuclear leukocytes. Formation of a novel dihydroxyeicosatetraenoic acid. J Biol Chem 254: 2643-2646

Borgeat P, Samuelsson B (1979b) Arachidonic acid metabolism in polymorphonuclear leukocytes: effects of ionophore A23187. Proc Natl Acad Sci 76: 2148-2152

Borgeat P, Samuelsson B (1979c) Metabolism of arachidonic acid in polymorphonuclear leukocytes: structural analysis of novel hydroxylated compounds. J Biol Chem 254: 7865-7869

Borgeat P, Samuelsson B (1979d) Arachidonic acid metabolism in polymorphonuclear leukocytes: unstable intermediate in formation of dihydroxy acids. Proc Natl Acad Sci USA 76: 3213-3217

Borgeat P, Sirois P (1981) Leukotrienes: a major step in the understanding of immediate hypersensitivity reactions. J Med Chem 24: 121-126

Brink C, Duncan PG, Douglas JS (1981a) Histamine, endogenous prostaglandins and cyclic nucleotides in the regulation of airway muscle responses in the guinea pig. Prostaglandins 22: 729-738

Brink C, Duncan PG, Douglas JS (1981b) The response and sensitivity to histamine of respiratory tissues from normal and ovalbumin-sensitized guinea pigs: effect of cyclooxygenase and lipoxygenase inhibition. J Pharmacol Exp Ther 217: 592-601

Brink C, Grimaud C, Guillot C, Orehek J (1980) The interaction between indomethacin and contractile agents on human isolated airway muscle. Br J Pharmac 69: 383-388

Burka JF (1981) The products of the lipoxygenase pathway of arachidonic acid metabolism. New Eng Soc Allergy Proc 2: 62-67

Burka JF (1983) Arachidonic acid metabolites and smooth muscle. In Biochemistry of smooth muscle. Ed Stephens NL. Published by CRC Press Inc. Florida pp. 141-168

Burka JF (1983a) Effects of selected bronchodilators on antigen- and A23187-induced contraction of guinea pig trachea. J Pharmacol Exp Ther 225: 427-435

Burka JF (1983b) Inhibition of antigen and calcium ionophore A23187 induced contractions of guinea pig airways by isoprenaline and forskolin. Can J Physiol Pharmacol 61: 581-589

Burka JF (1983c) Effects of calcium channel blockers and a calmodulin antagonist on contraction of guinea pig airways. European J Pharmacol 99: 257-268

Burka JF (1984) Inhibition of antigen- and calcium ionophore A23187-induced contractions of guinea pig isolated airways with 8-(Diethylamino)Octyl-3,4,5-Trimethoxybenzoate Hydrochloride (TMB-8) Int Archs Allergy Appl Immun 74: 362-364

Burka J, Ali M, McDonald J, Paterson N (1981) Immunological and non-immunological synthesis and release of prostaglandins and thromboxanes from isolated guinea pig trachea. Prostaglandins 22: 683-691

Burka JF, Eyre P (1974) The immunological release of slow-reacting substance of anaphylaxis from bovine lung. Can J Physiol Pharmacol 52: 1201-1204

Burka JF, Flower RJ (1974) Effects of modulators of arachidonic acid metabolism on the synthesis and release of slow-reacting substance of anaphylaxis. Br J Pharmac 65: 35-41

Burka JF, Flower RJ (1979) Effect of modulators of arachidonic acid metabolism on the synthesis and release of slow reacting substance of anaphylaxis. Br J Pharmacol 65: 35-41

Burka JF, Paterson NAM (1980) Evidence for lipoxygenase pathway involvement in allergic tracheal contraction. Prostaglandins 19: 499-515

Burka JF, Paterson NAM (1981) A comparison of antigen-induced and calcium ionophore A23187 induced contraction of isolated guinea pig trachea. Can J Physiol Pharmacol 59: 1031-1038

Burka JF, Paterson NAM (1981) The effects of SRS-A and histamine antagonists on antigen-induced contraction of guinea-pig trachea. European J Pharmacol 70: 489-499

Bray MA (1983) The pharmacology and pathophysiology of leukotriene B_4. Br Med Bull 39: 249-254

Brocklehurst WE (1960) The release of histamine and formation of a slow-reacting substance (SRS-A) during anaphylactic shock. J Physiol (Lond) 151: 416-435

Brostrom CO, Wolff DJ (1981) Properties and functions of calmodulin. Biochem Pharmacol 30: 1395-1405

Bruns RF, Thomsen WJ, Pugsley TA (1983) Binding of leukotriene C_4 and D_4 to membranes from guinea pig lung: regulation by ions and guanine nucleotides. Life Sci 33: 645-653

Bryant DH, Burns MW, LaZarrs L (1973) New type of allergic asthma due to IgG "reaginic" antibody. Br Med J 4: 589-592

Bryant DH, Burns MW, LaZarrs L (1975) Identification of IgG antibody as a carrier of reaginic activity in asthmatic patients. J Allergy Clin 56: 417-428

Burnstock G (1972) Purinergic nerves. Pharmacol Rev 24: 509-581

Cade JF, Clancy RL, Walker SE, Pain MCF (1981) Slow-reacting substance from alveolar macrophages - a mechanism of asthma? AJEBAK 59: 449-454

Chand N, DeRoth L (1979) Responses of guinea-pig lung parenchymal strips to prostaglandins and some selected autacoids. J Pharm Pharmacol 31: 712-714

Chand N, Eyre P (1978) The Schultz-Dale reaction: a review. Agents and Actions 8: 171-184

Cheng JB, Lang D, Bewtra A, Townley RG (1985) Tissue distribution and functional correlation of [^{3}H] leukotriene C_4 and [^{3}H] leukotriene D_4 binding sites in guinea-pig uterus and lung preparations. J Pharmacol Exp Ther 232: 80-87

Cheng JB, Townley RG (1982) Comparison of muscarinic and beta adrenergic receptors between bovine peripheral lung and tracheal smooth muscles: a striking difference in the receptor concentration. Life Sci 30: 2079-2086

Cheng JB, Townley RG (1982) Effects of chronic histamine and ovalbumin aerosols on pulmonary beta adrenergic in sensitized guinea pigs. Res Comm Chem Pathol Pharmacol 36: 507-510

Cheng JB, Townley RG (1984a) Identification of leukotriene D_4 receptor binding sites in guinea pig lung homogenates using [^{3}H] leukotriene D_4. Biochem Biophys Res Comm 118: 20-26

Cheng JB, Townley RG (1984b) Effect of the serine-borate complex on the relative ability of leukotriene C_4, D_4 and E_4 to inhibit lung and brain [^{3}H] leukotriene and [^{3}H] leukotriene C_4 binding: demonstration of the agonists' potency order for leukotriene D_4 and leukotriene C_4 receptors. Biochem Biophys Res Comm 119: 612-617

Cheng JB, Townley RG (1984c) Evidence for a similar receptor site for binding of [^{3}H] leukotriene E_4 and [^{3}H] leukotriene D_4 to the guinea-pig crude lung membrane. Biochem Biophys Res Comm 122: 949-954

Cheung WY (1980) Calmodulin plays a pirotal role in cellular regulation. Science 207: 19-27

Chiou CY, Malagodi MH (1975) Studies on the mechanism of action of a new Ca^{2+} antagonist, 8-(N,N-diethylamino) octyl 3,4,5-trimethoxybenzoate hydrochloride in smooth and skeletal muscles. Br J Pharmacol 53: 279-285

Clark MA, Cook M, Mong S, Hogaboom GK, Shorr R, Stadel J, Crooke ST (1984) Leukotriene C_4 ([^{3}H]-LTC_4) binding to membranes isolated from a hamster smooth muscle cell line (DDT1MF2). Life Sci 35: 441-448

Clayton DE, Busse WW, Buckner CK (1981) Contribution of vascular smooth muscle to contractile responses of guinea pig isolated lung parenchymal strips. Eur J Pharmacol 70: 311-320

Coles SJ, Said SI, Reid LM (1981) Inhibition by vasoactive intestinal peptide of glycoconjugate and lysozyme secretion by human airways in vitro. Am Rev Respir Dis 124: 531-536

Conolly ME, Greenacre JK (1976) The lymphocytes beta-adrenoceptor in normal subjects and patients with bronchial asthma. The effect of different forms of treatment on receptor function. J Clin Invest 58: 1307-1313

Constantine JW (1965) The spirally cut tracheal strip preparation. J Pharm Pharmacol 17: 384

Cooper DMF, Londos C, Rodbell M (1980) Adenosine receptor-mediated inhibition of rat cerebral cortical adenylate cyclase by a GTP-dependent process. Mol Pharmacol 18: 598-601

Copas JL, Borgeat P, Gardiner PJ (1982) The actions of 5-, 12-, and 15-HETE on tracheobronchial smooth muscle. Prostaglandins, Leukotrienes and Medicine 8: 105-114

Corey EJ (1982) Chemical studies on slow reacting substances/ leukotrienes. Experientia 38: 1259-1275

Creese BR, Bach MK (1983) Hyperreactivity of airway smooth muscle produced in vitro by leukotrienes. Prostaglandins, Leukotrienes and Medicine 11: 161-169

Creese BR, Denborough MA (1981) Sources of calcium for contraction of guinea pig isolated tracheal smooth muscle. Clin Exp Pharmacol Physiol 8: 175-182

Cushley MJ, Tattersfield AE, Holgate ST (1983) Inhaled adenosine and guanosine on airway resistance in normal and asthmatic subjects. Br J Clin Pharmacol 15: 161-165

Cushley MJ, Tattersfield AE, Holgate ST (1984) Adenosine-induced bronchoconstriction in asthma. Am Rev Respir Dis 129: 380-384

Cutz E, Chan W, Track NS, Goth A, Said SI (1978) Release of vasoactive intestinal polypeptide in mast cells by histamine hibernators. Nature 275: 661-662

Dahlen SE, Hansson G, Hedqvist P, Bjorck T, Granstrom E, Dahlen B (1983) Allergen challenge of lung tissue from asthmatics elicits bronchial contraction that correlates with the release of leukotrienes C_4, D_4 and E_4. Proc Natl Acad Sci USA 80: 1712-1716

Dalc HH, Laidlaw PP (1910) The physiological action of β-iminizol-ethylamine. J Physiol 41: 318-344

Dey RD, Said SI (1980) Immunocytochemical localization of VIP-immunoreactive nerves in bronchial walls and pulmonary vessels. Fed Proc 39: 1062

Diamond J (1978) Role of cyclic nucleotides in control of smooth muscle contraction. Ady Cyclic Nucleotide Res 9: 327-340

Diamond L, Richardson JB (1982) Inhibitory innervation to airway smooth muscle. Exp Lung Res 3: 379-385

Drazen JM, Austen KF, Lewis RA, Clark DA, Goto G, Marfat A, Corey EJ (1980) Comparitive airway and vascular activities of leukotrienes C-1 and D in vivo and in vitro. Proc Natl Acad Sci USA 77: 4354-4358

Drazen JM, Venugopalan CS, Austen KF, Brion F, Corey EJ (1982) Effects of leukotriene E on pulmonary mechanics in the guinea pig. Am Rev Respir Dis 125: 290-294

Drazen JM, Schneider MW (1978) Comparative responses of tracheal spirals and parenchymal strips to histamine and carbachol in vitro. J Clin Invest 61: 1441-1447

Ehlert FJ, Roeske WR, Rosenberger LB, Yamamura HI (1980) The influence of guanyl-5'-yl-imidodiphosphate and sodium on muscarinic receptor binding in the rat brain and longitudinal muscle of the rat ileum. Life Sci 26: 245-252

Engineer DM, Neiderhauser U, Piper PJ, Sirois P (1978) Release of mediators of anaphylaxis: inhibition of prostaglandin synthesis and the modification of release of slow reacting substance of anaphylaxis and histamine. Br J Pharmac 62: 61-66

Eriksson NE (1978) Food sensitivity reported by patients with asthma and hay fever. Allergy 33: 189-196

Eyre P, Burka JF (1978) Hypersensitivity in cattle and sheep - a pharmacological review. J Vet Pharmacol Therap 1: 97-109

Falkenheim SF, MacDonald H, Huber MM, Koch D, Parker CW (1980) Effect of the 5-hydroperoxide of eicosatetraenoic acid and inhibitors of the lipoxygenase pathway on the formation of slow reacting substance by rat basophilic leukemia cells; direct evidence that slow reacting substance is a product of the lipoxygenase pathway. J Immunol 125: 163-168

Fanta CH, Drazen JM (1983) Calcium blockers and bronchoconstriction. Am Rev Resp Dis 127: 673-674

Fanta CH, Venugopolan CS, Lacouture PG, Drazen JM (1982) Inhibition of bronchoconstriction in the guinea pig by a calcium channel blocker, nifedipine. Am Rev Respir Dis 125: 61-66

Farley JM, Miles PR (1978) The sources of calcium for acetylcholine-induced contraction of dog tracheal smooth muscle. J Pharmacol Exp Ther 207: 340-346

Farmer JB, Farrar DG, Wilson J (1974) Antagonism of tone and prostaglandin-mediated responses in a tracheal preparation by indomethacin and SC-19220. Br J Pharmac 52: 559-565

Feldberg W, Kellaway CH (1938) Liberation of histamine and formation of lysolecithin-like substances by cobra venom. J Physiol 94: 187-226

Fish JE, Ankin MG, Adkinson NF, Peterman VI (1981) Indomethacin modification of immediate-type immunologic airway responses in allergic asthmatic and non-asthmatic subjects. Evidence for altered arachidonic acid metabolism in asthma. Am Rev Respir Dis 123: 609-614

Fish JE, Jameson LS, Albright A, Norman PS (1984) Modulation of the bronchomotor effects of chemical mediators by prostaglandin $F_{2\alpha}$ in asthmatic subjects. Am Rev Respir Dis 130: 571-574

Fleisch JH, Haisch KD, Spaethe SM (1982) Slow reacting substance of anaphylaxis (SRS-A) release from guinea pig lung parenchyma during antigen- or ionophore-induced contraction. J Pharmacol Exp Ther 221: 146-151

Fleisch JH, Rinkema LE, Baker SR (1982) Evidence for multiple leukotriene D_4 receptors in smooth muscle. Life Sci 31: 577-581

Ford-Hutchinson AW, Bray MA, Doig MV, Shipley ME, Smith MJH (1980) Leukotriene B_4, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. Nature 286: 264-266

Foreman J (1980) The pharmacological control of immediate hypersensitivity. Ann Rev Pharmacol Toxicol 21: 63-81

Foreman J (1980) Receptor-secretion coupling in mast cells. TIPS 1: 460-463

Foreman J, Hallet M, Mongar J (1977) The relationship between histamine secretion and calcium uptake by mast cells. J Physiol 271: 193-214

Foreman J, Lichtenstein L (1980) Clinical pharmacology of acute allergic disorders. Ann Rev Med 31: 181-190

Fransden EK, Krishna GA, Said SI (1978) Vasoactive intestinal polypeptide promotes cyclic adenosine 3',5'-monophosphate accumulation in guinea pig trachea. Br J Pharmac 62: 367-369

Freeman DJ, Daniel EE (1973) Calcium movement in vascular smooth muscle and its detection using lanthanum as a tool. Can J Physiol Pharmacol 51: 900-913

Fruteau de Laclos B, Braquet P, Borgeat P (1984) Characterization of leukotriene and hydroxyeicosatetraenoic acid synthesis in human leukocytes in vitro: effect of arachidonic acid concentration. Prostaglandins, Leukotrienes and Medicine 13: 47-52

Furukawa M, Yoshimoto T, Ochi K, Yamamoto S (1984) Studies on arachidonate 5-lipoxygenase of rat basophilic leukemia cells. Biochem Biophys Acta 795: 458-465

Gardiner PJ, Collier HOJ (1980) Specific receptors for prostaglandins in airways. Prostaglandins 19: 819-841

Garland LG (1984) The pharmacology of airway hyperreactivity. TIPS 5: 338-340

Gavish M, Goodman RR, Snyder SH (1982) Solubilized adenosine receptors in the brain: regulation by guanine nucleotides. Science 215: 1633-1635

Ghelani AM, Holroyde MC, Sheard P (1980) Responses of human isolated bronchial and lung parenchymal strips to SRS-A and other mediators of asthmatic bronchospasm. Br J Pharmac 71: 107-112

Goetzl E (1981) Oxygenation products of arachidonic acid as mediators of hypersensitivity and inflammation. Medical Clinics of North America 65: 809-828

Gold WM, Kesller GF, Yu DYC (1972) Role of vagus nerves in experimental asthma in allergic dogs. J Appl Physiol 33: 719-725

Goodman FR (1981) Calcium-channel blockers and respiratory smooth muscle. In: New perspectives on calcium antagonists, Ed Weiss GB. Amer Physiol Soc, Bethesda MD pp. 217-222

Griffin M, Weiss JW, Leitch AG, McFadden ER Jr, Corey EJ, Austen KF,Drazen JM (1983) Effects of leukotriene D on the airways in asthma. New Eng J Med 308: 436-439

Grodzinska L, Panczenko B, Gryglewski RJ (1975) Generation of prostaglandin E-like material by the guinea pig trachea contracted by histamine. J Pharm Pharmacol 27: 88-91

Grundstrom N, Andersson RGG, Wikberg JES (1981) Pharmacological characterization of the autonomous innervation of the guinea pig tracheobronchial smooth muscle. Acta pharmacol et toxicol 49: 150-157

Hamasaki Y, Mojarad M, Saga T, Tai H, Said SI (1984) Platelet-activating factor raises airway and vascular pressures and induces edema in lungs perfused with platelet free solution. Am Rev Respir Dis 129: 742-746

Hamberg M, Samuelsson B (1974) Prostaglandin endoperoxides. VII Novel transformation of arachidonic acid in guinea pig lung. Biochem Biophys Res Comm 61: 942-949

Hammarstrom S (1983) Biological chemistry of the leukotrienes. In: Leukotrienes and other lipoxygenase products. Ed Piper PJ. Published by Research Studies Press Herts England pp. 1-14

Hammarstrom S, Murphy RC, Samuelsson B, Clark DA, Mioskowski C, Corey EJ (1979) Structure of leukotriene C. Identification of the amino acid part. Biochem Biophys Res Commun 91: 1266-1272

Hanna CJ, Bach MK, Pare PD, Schellenberg RR (1981) Slow reacting substances (leukotrienes) contract human airway and pulmonary vascular smooth muscle in vitro. Nature 290: 343-344

Hardman JG (1981) Cyclic nucleotides and smooth muscle contraction: some conceptual and experimental considerations. In: Smooth muscle: an assessment of current knowledge. Eds Bulbring E, Brading AF, Jones AW, Tomita T. Published by Edward Arnold, London, pp. 249-262

Hardy C, Robinson C, Lewis RA, Tattersfield AE, Holgate ST (1985) Airway and cardiovascular responses to inhaled prostacyclin in normal and asthmatic subjects. Am Rev Respir Dis 131: 18-21

Hargreave FE, Ryan G, Thomson NC, O'Byrne PM, Latimer K, Juniper EF, Dolovich J (1981) Bronchial responsiveness to histamine or methacholine in asthma: measurement and clinical significance. J Allergy Clin Immunol 68: 347-355

Hedqvist P, Dahlen E, Gustafsson L, Hammarstrom S, Samuelsson B (1980) Biological profile of leukotrienes C_4 and D_4. Acta Physiol Scand 110: 331-333

Hedman SE, Andersson RGG (1982) The cyclic AMP system in sensitized and desensitized guinea-pig tracheal smooth muscle. European J Pharmacol 83: 107-112

Hedman SE, Andersson RGG (1982) Effects of SRS (Slow Reacting Substance) on cyclic nucleotides in guinea pig tracheal muscle. Acta pharmacol et toxicol 50: 30-34

Henderson AF, Heaton RW, Dunlop LS, Costello JF (1983) Effects of nifedipine on antigen-induced bronchoconstriction. Am Rev Respir Dis 127: 549-553

Hendrick DJ, Hammad YY, Salvaggio JE (1981) Air pollution and asthma In: Bronchial asthma - principles of diagnosis and treatment. Ed Gershwin ME, published by Grune and Stratton Inc. USA pp. 235-255

Herrera H, Fialkor J (1981) Psychologic considerations in the evolution and natural history of bronchial asthma - In: Bronchial asthma principles of diagnosis and treatment. Ed Gershwin ME, published by Grune and Stratton Inc. USA pp. 405-426

Hildebrandt JD, Skeura RD, Codina J, Iyengar R, Manclark CR, Birnbaumer L (1983) Stimulation and inhibition of adenylyl cyclases mediated by distinct regulatory proteins. Nature 302: 706-709

Hill DR, Bowery NG (1981) ^{3}H-baclofen and ^{3}H-Gaba bind to biculline-insensitive $Gaba_B$ sites in rat brain. Nature 290: 149-152

Hirata F, Axelrod J (1980) Phospholipid methylation and biological signal transmission. Science 209: 1082-1090

Hirata F, Schiffmann E, Venkatasubranian K, Salomon D, Axelrod J (1980) A phospholipase A_2 inhibitory protein in rabbit neutrophils induced by glucocorticoids. Proc Natl Acad Sci USA 77: 2533-2536

Hoffman BB, Mulliken-Kilpatrick D, Lefkowitz RJ (1980) Heterogeneity of radioligand binding to alpha-adrenergic receptor: analysis of guanine nucleotide regulation of agonist binding in relation to receptor subtypes. J Biol Chem 255: 4645-4649

Hogaboom GK, Emler CA, Butcher FR, Fedan JS (1982) Concerted phosphorylation of endogenous tracheal smooth muscle membrane proteins by Ca^{2+}. Calmodulin, cyclic GMP- and cyclic AMP-dependent protein kinases. Febs Letters 139: 309-312

Hogg JC (1983) Pathology of asthma in allergy, principles and practice. Ed Middleton E Jr. et al. Published by C.V. Mosby St. Louis pp. 833-841

Holgate ST, Maan JS, Cushley MJ (1984) Adenosine as a broncho-constrictor mediator in asthma and its' antagonism by methylxanthines. J Allergy Clin Immunol 74: 302-306

Hughes PJ, Holgate ST, Church MK (1984) Adenosine inhibits and potentiates IgE-dependent histamine release from human lung mast cells by an A_2 purinoceptor mediated mechanism. Biochem Pharmacol 33: 3847-3852

Hulting A, Lingren J, Hokfelt T, Heidvall K, Eneroth P, Werner S, Patrono C, Samuelsson B (1985) Leukotriene C_4 stimulates LH secretion from rat pituitary cells in vitro. European J Pharmacol 106: 459-460

Hutas I, Hadhazy P, Debreczeni L, Vizi ES (1981) Relaxation of human isolated bronchial smooth muscle. Role of prostacyclin and prostaglandin $F_{2\alpha}$ in muscle tone. Lung 159: 153-161

Irvine RF (1982) How is the level of free arachidonic acid controlled in mammalian cells? Biochem J 204: 3-16

Ishizaka K (1976) Cellular events in the IgE antibody response. Adv Immunol 23: 1-75

Ishizuka K (1982) Possible approaches to turn off the IgE response Annals of Allergy 48: 320-324

Ishizaka T (1982) IgE and mechanisms of IgE-mediated hypersensitivity. Annal of Allergy 48: 313-319

Ishizaka T, Conrad DH, Schulman ES, Sterk AR, Ishizaka K (1983) Biochemical analysis of initial triggering events of IgE-mediated histamine release from human lung mast cells. J Immunol 130: 2357-2362

Ishizaka T, Conrad DH, Schulman ES, Sterk AR, Ko CGL, Ishizaka K (1984) IgE-mediated triggering signals for mediator release from human mast cells and basophils. Federation Proceedings 43: 2840-2845

Ishizuka Y, Imai A, Nozawa Y (1984) Polyphosphoinositide turnover in rat mast cells stimulated by antigen: rapid and preferential breakdown of phosphatidyl inositol 4-phosphate (DPI). Biochem Biophys Res Comm 123: 875-881

Ishizaka T, Hirata F, Ishizaka K, Axelrod J (1980) Stimulation of phospholipid methylation, calcium influx and histamine release by bridging of IgE receptors on rat mast cells. Proc Natl Acad Sci 77: 1903-1906

Iwayama Y, Chung CZ, Takayangi I (1982) Effects of anti-asthmatic drugs on airway resistance and plasma level of cyclic AMP in guinea pig. Japan J Pharmacol 32: 329-334

Jakobs KH, Schultz G (1980) Actions of hormones and neurotransmitters at the plasma membrane: inhibition of adenylate cyclase. TIPS 12: 331-333

Jakobs KH, Aktories K, Schultz G (1981) Inhibition of adenylate cyclase by hormones and neurotransmitters. Ad Cyc Nucleot Res 14: 173-187

Jakobs KH, Aktories K, Schulz G (1984) Mechanisms and components involved in adenylate cyclase inhibition by hormones in advances in cyclic nucleotide and protein phosphorylation research. Vol. 17. Ed Greengard P et al. Raven Press, N.Y. pp. 135-143

Jakschik BA, Harper T, Murphy RC (1982) Leukotriene C_4 and D_4 formation by particulate enzymes. J Biol Chem 257: 5346-5349

Jakschik BA, Lee LH (1980) Enzymatic assembly of slow reacting substance. Nature 287: 51-53

Johansson SGO, Bennich HH (1982) The clinical impact of the discovery of IgE. Annals of Allergy 48: 325-330

Jones TR, Davis C, Daniel EE (1982) Pharmacological study of the contractile activity of leukotriene C_4 and D_4 on isolated human airway smooth muscle. Can J Physiol Pharmacol 60: 638-643

Jorg A, Henderson WR, Murphy RC, Klebanoff SJ (1982) Leukotriene generation by eosinophils. J Exp Med 155: 390-402

Kaliner M, Shelhamer JH, Davis PB, Smith LJ, Venter JC (1982) Autonomic nervous system abnormalities and allergy. Ann Int Med 96: 349-357

Katsuki S, Murad F (1977) Regulation of adenosine cyclic 3',5'-monophosphate and guanosine cyclic 3',5'-monophosphate levels and contractility in bovine tracheal smooth muscle. Mol Pharmacol 13: 330-341

Kazimierczak W, Diamant B (1978) Mechanisms of histamine release in anaphylactic and anaphylactoid reactions. Prog Allergy 24: 295-365

Kellaway CH, Trethewie FR (1940) The liberation of a slow-reacting smooth muscle - stimulating substance in anaphylaxis. J Exp Physiol 30: 121-145

Kerr JW, Govindara JMI, Patel KR (1970) Effect of alpha-receptor blocking drugs and disodium cromoglycate on histamine hypersensitivity in bronchial asthma. Br Med J 2: 139-141

Koski G, Streaty RA, Klee WA (1982) Modulation of sodium sensitive GTPase by partial opiate agonist. J Biol Chem 257: 14035-14040

Krell RD, Osborn R, Vickery L, Falcone K, O'Donnell M, Gleason J, Kinzig C, Bryan D (1981) Contraction of isolated airway smooth muscle by synthetic leukotrienes C_4 and D_4. Prostaglandins 22: 387-409

Kreutner WR, Chapman A, Gulbenkion A, Tozzi S (1984) Bronchodilator and antiallergy activity of forskolin. J Allergy Clin Immun 73: 130

Krilis S, Lewis RA, Corey EJ, Austen KF (1983) Specific receptors for leukotriene C_4 on a smooth muscle cell line. J Clin Invest 72: 1516-1519

Krilis S, Lewis RA, Corey EJ, Austen KF (1984) Specific binding of leukotriene C_4 to ileal segments and subcellular fractions of ileal smooth muscle cells. Proc Natl Acad Sci USA 81: 4529-4533

Kuehl FA Jr., Dougherty HW, Ham EA (1984) Interactions between prostaglandins and leukotrienes. Biochem Pharmacol 33: 1-5

Kukovetz WR, Holzmann S, Wurm A, Poch G (1979) Evidence for cyclic GMP-mediated relaxant effects of nitro-compounds in coronary smooth muscle. Naunyn-Schmiedebergs Arch Pharmac 310: 129-138

Kuo CG, Lewis MT, Jakschik BA (1984) Leukotriene D_4 and E_4 formation by plasma membrane bound enzymes. Prostaglandins 28: 929-938

Lagunoff D (1983) The role of mast cells in asthma. Exp Lung Res 4: 121-135

Lapetina EG, Billah MM, Cuatrecasas P (1981) The phosphatidyl inosital cycle and the regulation of arachidonic acid production. Nature 292: 367-369

Lau YS, Lum BKB (1983) Roles of cyclic AMP in adrenergically-induced tracheal muscle relaxation. Archives Internationales de Pharmacodynamic et de Therapie 261: 36-50

Lee TH, Austen KF, Corey EJ, Drazen JM (1984) Leukotriene E_4-induced airway hyperresponsiveness of guinea pig tracheal smooth muscle to histamine and evidence for three separate sulfidopeptide leukotriene receptors. Proc Natl Acad Sci USA 81: 4922-4925

Lefer AM, Lepran I, Roth DM, Smith JB (1984) Specificity of anti-leukotriene actions of nicardipine. Pharmacol Res Commun 16: 1141-1150

Leff A (1982) Pathogenesis of asthma. Chest 81: 224-229

Lefkowitz RJ, Michel T (1983) Plasma membrane receptors. J Clin Invest 72: 1185-1189

Lefkowitz RJ, Stadel JM, Caron MG (1983) Adenylate cyclase coupled beta-adrenergic receptors: structure and mechanisms of activation and desensitization. Ann Rev Biochem 52: 159-186

Levine L, Morgan RA, Lewis RA, Austen KF, Clark DA, Marfat A, Corey EJ (1981) Radioimmunoassay of the leukotrienes of slow reacting substance of anaphylaxis. Proc Natl Acad Sci USA 78: 7692-7696

Lewicki JA, Brandwejn HJ, Mittal CK, Arnold WP, Murad F (1982) Properties of purified soluble guanylate cyclase activated by nitric oxide and sodium nitroprusside. J Cyclic Nuc Res 8: 17-25

Levitzki A (1982) Activation and inhibition of adenylate cyclase by hormones: mechanistic aspects. TIPS May, pp. 203-208

Lewis RA, Austen KF (1981) Mediation of local homeostasis and inflammation by leukotrienes and other mast cell-dependent compounds. Nature 293: 103-108

Lewis RA, Austen KF, Drazen JM, Clark DA, Marfat A, Corey EJ (1980) Slow reacting substances of anaphylaxis: identification of leukotrienes C-1 and D from human and rat sources. Proc Natl Acad Sci USA 77: 3710-3714

Lindgren JA, Hokfelt T, Dahlen SE, Patrono C, Samuelsson B (1984) Leukotrienes in the rat central nervous system. Proc Natl Acad Sci USA 81: 6212-6216

Lewis MA, Mong S, Vessella RL, Hogaboom GK, Wu H, Crooke ST (1984) Identification of specific binding sites for leukotriene C_4 in human fetal lung. Prostaglandins 27: 961-974

Mathe AA, Yen S, Sohn R, Hedqvist P (1977) Release of prostaglandins and histamine from sensitized and anaphylactic guinea pig lungs - changes in cyclic AMP levels. Biochem Pharmacol 26: 181-188

Matsuzaki Y, Hamasaki Y, Said SI (1980) Vasoactive intestinal peptide: A possible transmitter of nonadrenergic relaxation of guinea pig airways. Science 210: 1252-1253

McGiff JC (1981) Prostaglandins, prostacyclin and thromboxanes. Ann Rev Pharmacol Toxicol 21: 479-509

McIntyre E, Fitzgibbon B, Otto H, Minson R, Alpers J, Ruffin R (1983) Inhaled verapamil in histamine-induced bronchoconstriction. J Allergy Clin Immunol 71: 375-381

Menzel DB, Roycroft JH, Nixon JR, Issac SR, Porter NA (1976) Monocyclic peroxides as inhibitors of arachidonic acid and prostaglandin endoperoxide analog initiated aggregation of human platelets. Res Commun Chem Pathol Pharmacol 15: 767-785

Metzger H (1979) Early molecular events in the antigen-antibody cell activation. Ann Rev Pharmacol Toxicol 19: 427-445

Meurs H, Koeter GH, de Vries K, Kauffman HF (1982) The beta-adrenergic system and allergic bronchial asthma: changes in lymphocyte beta-adrenergic receptor number and adenylate cyclase activity after an allergen-induced asthmatic attack. J Allergy Clin Immunol 70: 272-280

Michell R, Kirk C (1980) Why is phosphatidyl inosital degraded in response to stimulation of certain receptors? TIPS April: 86-89

Middleton E (1980) Antiasthmatic drug therapy and calcium ions: Review of pathogenesis and role of calcium. J Pharmaceutical Sci 69: 243-251

Middleton E (1984) Airway smooth muscle, asthma and calcium ions. J Allergy Clin Immunol 73: 643-650

Mitchell HW, Denborough MA (1979) Anaphylaxis in guinea-pig peripheral airways in vitro. European J Pharmacol 54: 69-78

Mitchell HW, Denborough MA (1980) The metabolism of arachidonic acid in the isolated tracheal and lung strip preparations of guinea pigs. Lung 158: 121-129

Molski TFP, Naccache PH, Marsh ML, Kermode J, Becker EL, Shaafi RI (1984) Pertussis toxin inhibits the rise in the intracellular concentration of free calcium that is induced by chemotactic factors in rabbit neutrophils: possible role of the "G proteins" in calcium mobilization. Biochem Biophys Res Commun 124:644-650

Moncada S, Vane JR (1979) Pharmacology and endogenous roles of prostaglandin endoperoxides, thromboxane A_2, and prostacyclin. Pharmacol Revs 30: 293-331

Mong S, Wu H, Hogaboom GK, Clark MA, Crooke ST (1984a) Characterization of the leukotriene D_4 receptor in guinea-pig lung. European J Pharmacol 102: 1-11

Mong S, Wu H, Clark MA, Stadel JM, Gleason JG, Crooke ST (1984b) Identification of leukotriene D_4 specific binding sites in the membrane preparation isolated from guinea pig lung. Prostaglandins 28: 805-822

Mong S, Wu H, Hogaboom GK, Clark MA, Stadel JM, Crooke ST (1985) Regulation of ligand binding to leukotriene D_4 receptors: effects of cations and guanine nucleotides. European J Pharmacol 106: 241-253

Mongar JL (1965) Mechanism of passive sensitization. Arch Exp Pathol Pharmakol 250: 124-135

Morcillo EJ, Perpina M, Esplugues J (1984) Hyperresponsiveness to autacoids and autonomic drugs in lung parenchymal strips from sensitized guinea pigs. Am Rev Respir Dis 129: 948-951

Morris HR, Piper PJ, Taylor GW, Tippins JR (1979) The effect of arachidonate lipoxygenase substrates and inhibitors on SRS-A release in guinea pig lung. Br J Pharmacol 66: 452

Morris HR, Taylor GW, Jones CM, Piper PJ, Samhoun MN, Tippins JR (1982) Slow reacting substances (leukotrienes): enzymes involved in their biosynthesis. Proc Natl Acad Sci USA 79: 4838-4842

Morris HR, Taylor GW, Piper PJ, Samhoun MN, Tippins JR (1980a) Slow reacting substances (SRSs): The structure identification of SRSs from rat basophilic leukaemia (RBL-1) cells. Prostaglandins 19: 185-201

Morris HR, Taylor GW, Piper PJ, Sirois P, Tippins JR (1978) Slow reacting substance of anaphylaxis - purification and characterization. Febs Lett 87: 203-206

Morris HR, Taylor GW, Piper PJ, Tippins JR (1980) Structure of slow-reacting substance of anaphylaxis from guinea-pig lung. Nature 285: 104-106

Morris HR, Taylor GW, Piper PJ, Tippins JR (1980b) Structure of slow-reacting substance of anaphylaxis from guinea-pig lung. Nature 285: 104-106

Motulsky HJ, Insel PA (1983) Influence of sodium on the α_2-adrenergic receptor system of human platelets, role of intraplatelet sodium in receptor binding. J Biol Chem 258: 3913-3917

Muller MJ (1985) Ph.D. Thesis. University of Alberta

Murad F (1974) Mechanism of action of some bronchodilators. Cyclic nucleotide metabolism in tracheal preparations. Am Rev Resp Dis 110: 111-118

Murlas C, Nadel JA, Roberts JM (1982) The muscarinic receptors of airway smooth muscle: their characterization in vitro. J Appl Physiol 52: 1084-1091

Murphy RC, Hammarstrom S, Samuelsson B (1979) Leukotriene C. A slow reacting substance (SRS) from murine mastocytoma cells. Proc Natl Acad Sci USA 76: 4275-4279

Nadel JA, Barnes PJ (1984) Autonomic regulation of the airways. Ann Rev Med 35: 451-467

Nicosia S, Crowley HJ, Oliva D, Welton AF (1984) Binding sites for ^{3}H-LTC$_4$ in membranes from guinea pig ileal longitudinal muscle. Prostaglandins 27: 483-494

O'Byrne PM, Ryan G, Morris M, McCormack D, Jones NL, Morse JLC, Hargreave FE (1982) Asthma induced by cold air and its regulation to nonspecific bronchial responsiveness to methacholine. Am Rev Respir Dis 125: 281-285

O'Flaherty JT (1982) Biology of disease: lipid mediators of inflammation and allergy. Laboratory Investigation 47: 314-329

Ohkubo H, Takayanagi I, Takagi K (1976) Relationship between the levels of intracellular cyclic nucleotides and mechanical responses induced by drugs. Japan J Pharmacol 26: 65-71

Olianas MC, Onali P, Neff NH, Costa E (1982) Adenylate cyclase activity of synaptic membranes from rat striatum. Inhibition by muscarinic receptor agonists. Mol Pharmacol 23: 393-398

Omini C, Folco GC, Vigano T, Rossoni G, Brunelli G, Berti F (1981) Leukotriene C_4 induces generation of PGI_2 and TXA_2 in guinea-pig in vivo. Pharmacol Res Commun 13: 633-640

Orehek J (1982) Asthma without airway hyperreactivity: fact or artifact. Eur J Respir Dis 63: 1-4

Orehek J, Douglas JS, Bouhuys A (1975) Contractile responses of the guinea pig trachea in vitro: modification by prostaglandin synthesis inhibiting drug. J Pharmacol Exp Ther 194: 554-564

Orehek J, Gayrard P, Grimaud CH, Charpin J (1975) Effects of beta adrenergic blockade on bronchial sensitivity to inhaled acetylcholine in normal subjects. J Allergy Clin Immunol 55: 164-169

Palmer MR, Mathews WR, Hoffer BJ, Murphy RC (1981) Electrophysiological responses of cerebellar purkinje neurons to leukotriene D_4 and B_4. J Pharmacol Exp Ther 219: 91-96

Palmer MR, Mathews R, Murphy RC, Hoffer BJ (1980) Leukotriene C elicits a prolonged excitation of cerebellar purkinje neurons. Neuroscience Lett 18: 173-180

Patel KR (1981a) The effect of calcium antagonist, nifedipine in exercise-induced asthma. Clinical Allergy 11: 429-432

Patel KR (1981b) The effect of verapamil on histamine and methacholine-induced bronchoconstriction. Clinical Allergy 11: 441-447

Patel KR, Al Shama MR, Kerr JW (1983) The effect of inhaled verapamil on allergen-induced bronchoconstriction. Clinical Allergy 13: 119-122

Paterson NAM, Burka JF, Craig ID (1981) Release of slow-reacting substance of anaphylaxis from dispersed pig lung cells: effect of cyclo-oxygenase and lipoxygenase inhibitors. J Allerg Clin Immunol 67: 426-434

Pearce F, Ennis M, Truneh A, White J (1981) Role of intra- and extracellular calcium in histamine release from peritoneal mast cells. Agents and Actions II, 1/2 51-54

Peters SP, MacGloshan DW Jr, Schulman ES, Schleimer RP, Lichtenstein LM (1983) The production of arachidonic acid metabolites by purified human lung mast cells. Fed Proc 42: 1375-1381

Peters SP, Siegel MI, Kagey-Sobotka A, Lichtenstein LM (1981) Lipoxygenase products modulate histamine release in human basophils. Nature 292: 455-457

Phillips MJ, Gold WM, Goetzl EJ (1983) IgE-dependent and ionophore-induced generation of leukotrienes by dog mastocytoma cells. J Immunol 131: 906-910

Piper PJ (1983) Leukotrienes. Tips 4: 75-77

Piper PJ, Samhoun MN (1981) The mechanism of action of leukotrienes C_4 and D_4 in guinea-pig isolated perfused lung and parenchymal strips of guinea pig, rabbt and rat. Prostaglandins 21: 793-802

Piper PJ, Samhoun MN (1982) Stimulation of arachidonic acid metabolism and generation of thromboxane A_2 by leukotrienes B_4, C_4, and D_4 in guinea pig lung in vitro. Br J Pharmac 77: 267-275

Piper PJ, Seale JP (1979) Non-immunological release of slow-reacting substance from guinea-pig lungs. Br J Pharmac 67: 67-72

Piper PJ, Tippins JR, Morris HR, Taylor GW (1979) Arachidonic acid metabolism and SRS-A. Agents and Actions, Supplement 4: 37-48

Polson JB, Krzanowski JJ, Szentivanyi A (1982) Inhibition of a high affinity cyclic AMP phosphodiesterase and relaxation of canine tracheal smooth muscle. Biochem Pharmacol 31: 3403-3406

Pong S, DeHaven RN (1983) Characterization of a leukotriene D_4 receptor in guinea pig lung. Proc Natl Acad Sci USA 80: 7415-7419

Pong S, DeHaven RN, Kuehl FA Jr, Egan RW (1983) Leukotriene C_4 binding to rat lung membranes. J Biol Chem 258: 9616-9619

Raeburn D, Rodger IW (1984) Lack of effect of leukotriene D_4 on Ca-uptake in airway smooth muscle. Br J Pharmac 83: 499-504

Rankin JA, Hitchcock M, Merrill W, Bach MK, Brashler JR, Askenase PW (1982) IgE-dependent release of leukotriene C_4 from alveolar macrophages. Nature 297: 329-331

Rasmussen H, Barrett PQ (1984) Calcium messenger system: an integrated view. Physiological Rev 64: 938-984

Regal JF (1984) IgG vs IgE: Mediators of antigen-induced guinea pig tracheal contraction. Immunopharmacol 8: 111-119

Richardson JB (1979) Nerve supply to the lungs. Am Rev Respir Dis 119: 785-801

Richardson J (1981) Noradrenergic inhibitory innervation of the lung. Lung 159: 315-322

Richardson JB, Beland J (1976) Non-adrenergic inhibitory nerves in human airways. J Appl Physiol 41: 764-771

Richardson PS, Sterling GM (1969) Effects of β-adrenergic receptor blockade on airway conductance and lung volume in normal and asthmatic subjects. Br Med J 3: 143-145

Rinard GA, Jensen A (1981) Preparation of hormone sensitive airway smooth muscle adenylate cyclase from dissociated canine trachealis cells. Biochimica et Biophysica Acta 678: 207-212

Rinard GA, Jensen A, Puckett AM (1983) Hydrocortisone and isoproterenal effects on trachealis cAMP and relaxation. J Appl Physiol: Respirat Environ Exercise Physiol 55: 1609-1613

Rinard GA, Rubinfeld AR, Brunton LL, Mayer SE (1979) Depressed cyclic AMP levels in airway smooth muscle from asthmatic dogs. Proc Natl Acad Sci USA 76: 1472-1476

Robberecht P, Chatelain P, DeNeef P, Camus J, Waelbroeck M, Christophe J (1981) Presence of vasoactive intestinal peptide receptors coupled to adenylate cyclase in rat lung membranes. Biochem Biophys Acta 678: 76-82

Rodbell M (1980) The Role of hormone receptors and GTP-regulatory proteins in membrane transduction. Nature 284: 17-22

Saeed SA, Mitchell MD (1982) Arachidonate lipoxygenase activity in human fetal lung. Eur J Pharmac 78: 389-391

Said SI (1982) Vasoactive peptides in the lung with special reference to vasoactive intestinal peptides. Exp Lung Res 3: 343-348

Said SI, Kitamura S, Yoshida T, Preskitt J, Holden LD (1974) Hormonal control of airways. Ann NY Acad Sci 221: 103-114

Salmon DM, Honeyman TW (1980) Proposed mechanism of cholinergic action in smooth muscle. Nature 284: 344-345

Samhoun MN, Piper PJ (1984) Actions and interactions of lipoxygenase and cyclo-oxygenase products in respiratory and vascular tissues. Prostaglandins, Leukotrienes and Medicine 13: 79-87

Samuelsson B (1981) The leukotrienes: a novel group of compounds including SRS-A and mediators of inflammation. In: SRS-A and leukotrienes. Ed Piper PJ, published by John Wiley and Sons, Ltd. pp. 45-64

Sano Y, Watt G, Townley RG (1983) Decreased mononuclear cell beta-adrenergic receptors in bronchial asthma: parallel studies of lymphocyte and granulocyte desensitization. J Allergy Clin Immunol 72: 495-503

Schied CR, Honeyman TW, Fay FS (1979) Mechanism of β-adrenergic relaxation of smooth muscle. Nature 277: 32-36

Schellenberg RR, Foster A (1984) In vitro responses of human asthmatic airway and pulmonary vascular smooth muscle. Int Archs Allergy Appl Immun 75: 237-241

Schmidt K, Munshi R, Baer HP (1984) Characterization of forskolin binding sites in rat brain membranes using [14,15-^{3}H]14,15-dihydroforskolin as a ligand. Naunyn-Schmiedebergs Arch Pharmac 325: 153-158

Schneider MW, Drazen JM (1980) Comparative in vitro effects of arachidonic acid metabolites on tracheal spirals and parenchymal strips. Am Rev Respir Dis 121: 835-842

Schuhl JF, Pereyrn JG (1979) Oral acetylsalicylic acid (aspirin) challenge in asthmatic children. Clinical Allergy 9: 83-88

Schulman ES, Newball HH, Demers LM, Fitzpatrick FA, Adkinson NF Jr. (1981) Anaphylactic release of thromboxane A_2, prostaglandin D_2, and prostacyclin from human lung parenchyma. Am Rev Respir Dis 124: 402-406

Seamon K, Daly JW (1981a) Activation of adenylate cyclase by the diterpene forskolin does not require the guanine nucleotide regulatory protein. J Biol Chem 256: 9799-9801

Seamon KB, Daly JW (1981b) Forskolin: a unique diterpene activator of cyclic AMP-generating systems. J Cycl Nucl Res 7: 201-224

Seamon KB, Daly JW (1982) Guanosine 5'-(β,γ-imido) triphosphate inhibition of forskolin activated adenylate cyclase is mediated by the putative inhibitory guanine nucleotide regulatory protein. J Biol Chem 257: 11591-11596

Seamon KB, Padgett W, Daly JW (1981) Forskolin: unique diterpene activator of adenylate cyclase in membranes and in intact cells. Proc Natl Acad Sci USA 78: 3363-3367

Seamon KB, Vaillancourt R, Edwards M, Daly JW (1984) Binding of [^{3}H] forskolin to rat brain membranes. Proc Natl Acad Sci USA 81: 5081-5085

Shelhamer JH, Marom Z, Kaliner M (1983) Abnormal beta-adrenergic responsiveness in allergic subjects. II. The role of selective beta$_2$-adrenergic hyporeactivity. J Allergy Clin Immunol 71: 57-61

Sirois P, Borgeat P (1980) From slow reacting substance of anaphylaxis (SRS-A) to leukotrienes D_4 (LTD_4). Int J Immunopharmac 2: 281-293

Sirois P, Kerouac R, Roy S, Borgeat P, Picard S, Rioux F (1981) In vivo effects of leukotriene B_4, C_4, and D_4. Evidence that changes in blood pressure are mediated by prostaglandins. Prostaglandins and Medicine 7: 363-373

Sirois P, Roy S, Borgeat P, Picard S, Vallerand P (1982) Evidence for a mediator role of thromboxane A_2 in the myotropic action of leukotriene B_4 (LTB_4) on the guinea-pig lung. Prostaglandins, Leukotrienes and Medicine 8: 157-170

Sirois P, Roy S, Tetrault JP, Borgeat P, Picard S, Corey EJ (1981) Pharmacological activity of leukotrienes A_4, B_4, C_4 and D_4 on selected guinea-pig, rat, rabbit and human smooth muscles. Prostaglandins and Medicine 7: 327-340

Sly RM (1981) Hyperirritable airway syndrome - bronchial asthma: principles of diagnosis and treatment. Edited by Gershwin ME, published by Grune and Stratton Inc USA pp. 53-72

Snashall PD, Baldwin C (1982) Mechanism of sulphur dioxide induced bronchoconstriction in normal and asthmatic man. Thorax 37: 118-123

Snyder DW, Aharony D, Dobson P, Tsai BS, Krell RD (1984) Pharmacological and biochemical evidence for metabolism of peptide leukotrienes by guinea-pig airway smooth muscle in vitro. J Pharmacol Exp Ther 231: 224-229

Snyder DW, Krell RD (1984) Pharmacological evidence for a distinct leukotriene C_4 receptor in guinea-pig trachea. J Pharmacol Exp Ther 231: 616-622

So SY, Lam WK, Yu DYC (1982) Effects of calcium antagonists on allergen-induced asthma. Clinical Allergy 12: 595-600

Sok D, Pai J, Atrache V, Sih CJ (1980) Characterization of slow reacting substances (SRSs) of rat basophilic leukemia (RBL-1) cells: effect of cysteine on SRS profile. Proc Natl Acad Sci USA 77: 6481-6485

Sokolovsky M, Gurwitz D, Galron R (1980) Muscarinic receptor binding in mouse brain: regulation by guanine nucleotides. Biochem Biophys Res Comm 94: 487-492

Songsiridej V, Buckner C, Busse W (1982) Small airway contribution to antigen-induced contraction of isolated guinea pig lung parenchymal strips. J Allergy Clin Immunol 69: 107-111

Spannhake EW, Hyman AC, Kadowitz PJ (1981) Bronchoactive metabolites of arachidonic acid and their role in airway function. Prostaglandins 22: 1013-1026

Sparrow MP, Pfitzer G, Gagelmann M, Ruegg JC (1984) Effect of calmodulin, Ca^{2+}, and CAMP protein kinase on skinned tracheal smooth muscle. Am J Physiol 246: C308-C314

Speer F, Densison TR, Baptist JE (1982) Aspirin allergy. Annals of Allergy 46: 123-126

Stanescu DC, Frans A (1982) Bronchial asthma without increased airway reactivity. Eur J Respir Dis 63: 5-12

Steinhardt RA, Alderton JM (1982) Calmodulin confers calcium sensitivity on secretory exocytosis. Nature 295: 154-155

Stenson WF, Parker CW (1980) Prostaglandins, macrophages and immunity. J Immunol 125: 1-5

Stimler NP, O'Flaherty JT (1983) Spasmogenic properties of platelet-activating factor: evidence for a direct mechanism in the contractile response of pulmonary tissues. Am J Pathol 113: 75-84

Stull JT, Sanford CF (1981) Differences in skeletal, cardiac, and smooth muscle contractile element regulation by calcium. In: New perspectives on calcium antagonists. Ed Weiss GB. Amer Physiol Soc Bethesda MD pp. 35-46

Sullivan TJ, Parker CW (1976) Pharmacological modulation of inflammatory mediator release by rat mast cells. Am J Pathol 85: 437-464

Sullivan TJ, Parker KL, Kulczycki A, Parker CW (1976) Modulation of cyclic AMP in purified mast cells. III. Studies on the effects of concanavalin A and anti-IgE on cyclic AMP concentration during histamine release. J Immunol 117: 713-716

Sun FF, Chapman JP, McGuire JC (1977) Metabolism of prostaglandins, endoperoxides in animal tissues. Prostaglandins 14: 1055-1074

Sutherland EW, Rall TW (1960) The relation of adenosine-3'5'-phosphate and phosphorylase to the actions of catecholamines and other hormones. Pharmacol Rev 12: 265-299

Sydbom A, Fredholm BB (1982) On the mechanism by which theophylline inhibits histamine release from rat mast cells. Acta Physiol Scand 114: 243-251

Sydbom A, Fredholm BB, Uvnas B (1979) Effect of sensitization on spontaneous and phosphatidylserine-induced histamine release and on cyclic AMP and GMP levels in isolated rat mast cells. Acta Physiol Scand 106: 473-479

Szentivanyi A (1968) The beta-adrenergic theory of the atopic abnormality in bronchial asthma. J Allergy 42: 203-231

Tada T (1975) Regulation of reaginic antibody formation in animals. Prog Allergy 19: 122-194

Tate SS, Meister A (1978) Serine-borate complex as a transition-state inhibitor of y-glutamyl transpeptidase. Proc Natl Acad Sci USA 75: 4806-4809

Taylor GW, Morris HR (1983) Lipoxygenase pathways. Br Med Bull 39: 219-222

Theoharides TC, Bondy PK, Tsakalus ND, Askenase PW (1982) Differential release of serotonin and histamine from mast cells. Nature 297: 229-231

Tipton WR, Nelson HS, Souhrada JF, Morris HG, Jacobson KW (1981) Dynamics of isoproterenol subsensitivity in guinea pig airway smooth muscle. Lung 159: 199-210

Triggle DJ (1981) Calcium antagonists: basic chemical and pharmacological aspects. In: New perspectives in calcium antagonists. Ed Weiss GB. Amer Physiol Soc Bethesda MD pp. 1-18

Triggle DJ (1983) Calcium, the control of smooth muscle function and bronchial hyperreactivity. Allergy 38: 1-9

Tsai BS, Lefkowitz RJ (1979) Agonist specific effects of guanine nucleotides on alpha-adrenergic receptors in human platelets. Mol Pharmacol 16: 61-68

Tung RS, Lichtenstein LM (1981) Cyclic AMP agonist inhibition increases at low levels of histamine release from human basophils. J Pharmac Exp Ther 218: 642-646

Turker RK, Zengil H (1976) Release of prostaglandin-like material from isolated cat tracheal muscle by electrical and mechanical stimulation. Arch Int Physiol Biochem 84: 833-841

Uddman R, Alumets J, Densert O, Hakanson R, Sundler F (1978) Occurrence and distribution of VIP nerves in the nasal mucosa and tracheobronchial wall. Acta Otolaryngal (Stockh) 86: 443-448

Ukena D, Poeschla E, Schawbe U (1984) Guanine nucleotide and cation regulation of radioligand binding to Ri adenosine receptors of rat fat cells. Naunyn-Schmiedebergs Arch Pharmacol 326: 241-247

Undem BJ, Dick EC, Buckner CK (1983) Inhibition by vasoactive intestinal peptide of antigen-induced histamine release from guinea pig minced lung. European J Pharmac 88: 247-250

Vane JR (1971) Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature (London) 231: 232-235

Walters EH (1983) Effect of inhibition of prostaglandin synthesis on induced bronchial hyperresponsiveness. Thorax 38: 195-199

Walters EH, Bevon M, Davies BH (1982) The effects of oral propranolol and indomethacin on the response to inhaled PGE_2 in normal human subjects. Prostaglandins, Leukotrienes and Medicine 8: 141-150

Walters EH, Parrish RW, Bevan C, Smith AP (1981) Induction of bronchial hypersensitivity: evidence for a role for prostaglandins. Thorax 36: 571-574

Wanner A (1983) Allergic mucociliary dysfunction. J Allergy Clin Immunol 72: 347-350

Wasserman MA (1976) Bronchopulmonary pharmacology of some prostaglandin endoperoxide analogs in the dog. European J Pharmacol 36: 103-114

Webb RC, Bhalla RC (1976) Calcium sequestration by subcellular fractions isolated from vascular smooth muscle: effects of cyclic nucleotides and prostaglandins. J of Molecular and Cellular Cardiology 8: 145-157

Weber RW, Hoffman M, Raine DA, Nelson HS (1979) Incidence of bronchoconstriction due to aspirin, azo dyes, non-azo dyes, and preservatives in a population of perennial asthmatics. J Allergy Clin Immunol 64: 32-37

Webster S, Olsson RA (1981) Adenosine regulation of canine cardiac adenylate cyclase. Biochem Pharmacol 30: 369-373

Weichman BM, Hostelley LS, Bostick SP, Muccitelli RM, Krell RD, Gleason JG (1982) Regulation of the synthesis and release of slow-reacting substance of anaphylaxis from sensitized monkey lung. J Pharmacol Exp Ther 221: 295-302

Weichman BM, Muccitelli RM, Tucker SS, Wasserman MA (1983) Effect of calcium antagonists on leukotriene D_4-induced contractions of the guinea pig trachea and lung parenchyma. J Pharmacol Exp Ther 225: 310

Weichman BM, Tucker SS (1984) Leukotriene D_4 elicits a non-sustained contraction of the guinea pig trachea in calcium-free buffer. European J Pharmacol 101: 229-234

Weiss EB, Markowicz J, Barbero L (1982) Effect of calcium antagonists in experimental asthma. Allergy 37: 513-519

Weller PF, Lee CW, Foster DW, Corey EJ, Austen KF, Lewis RA (1983) Generation and metabolism of 5-lipoxygenase pathway leukotrienes by human eosinophils: predominant production of leukotrienes C_4. Proct Natl Acad Sci USA 80: 7626-7630

Welliver RC (1983) Upper respiratory infections in asthma. J Allergy Clin Immunol 72: 341-346

Wightman PD, Dahlgren ME, Bonney RJ (1982) Protein kinase activation of phospholipase A_2 in sonicates of mouse peritonal macrophages. J Biol Chem 257: 6650-6652

Wojick WJ, Neff NH (1983) γ-Amino butyric acid β receptors are negatively coupled to adenylate cyclase in brain, and in the cerebellum these receptors may be associated with granule cells. Mol Pharmacol 25: 24-28

Yamaguchi T, Hitzig B, Coburn RF (1976) Endogenous prostaglandins and mechanical tension in canine trachealis muscle. Am J Physiol 230: 1737-1743

Yen SS (1981) Inhibition of arachidonic acid-induced contraction of guinea pig lung strips. Prostaglandins 22: 183-194

Yen SS, Kreutner W (1980) Effect of inhibition of arachidonic acid metabolism on anaphylaxis of guinea pig lung strips. Agents and Actions 10: 274-277

Yen SS, Morris HG (1981) An imbalance of arachidonic acid metabolism in athma. Biochem Biophys Res Comm 103: 774-779

Yip P, Palombini B, Coburn RF (1981) Inhibitory innervation to the guinea pig trachealis muscle. J Appl Physiol: Respirat Environ Exercise Physiol 50: 374-382

Zavoico GB, Feinstein MB (1984) Cytoplasmic Ca^{2+} in platelets is controlled by cyclic AMP: Antagonism between stimulators and inhibitors of adenylate cyclase. Biochem Biophys Res Commun 120: 579-585